IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
HEATH, *et al.*
Serial No.: 08/699,716
Filed: 27 August 1996
For: RECOMBINANT F1-V PLAGUE VACCINE

Art Unit: 1645
Examiner: Duffy, Patricia Ann
Atty. Dckt: 003/029/SAP

AFFIDAVIT OF GEORGE W. ANDERSON, JR.

1. I, George W. Anderson, Jr., an inventor of the above-referenced application and resident of Smithsburg, MD, declare the following:
2. My curriculum vitae is attached.
3. Arthur M. Friedlander, David G. Heath, Susan L. Welkos and I are joint inventors of the subject matter disclosed in the above-referenced application.
4. From **[redacted date which is before 13 March 1996]** to February 1998, I conducted research and development on a plague vaccine comprising a F1-V fusion protein as an immunogen as part of the Army Plague Vaccine Group.
5. Before about **[redacted date which is before 13 March 1996]**, I obtained alhydrogel F1-V partial preparations from David G. Heath.
6. In my laboratory notebook I usually referred to the F1-V partial as "F1-V".
7. On **[redacted date which is before 13 March 1996]**, I began mouse challenge studies with the F1-V partial preparations I obtained from David G. Heath. The experimental protocol for the challenge studies is provided in my notebook #3598 on page 123. See Exhibit GA1.
8. The results dated **[redacted date which is before 13 March 1996]** for the challenge studies using *Yersinia pestis* strain CO92, which is $F1^+$ strain, with mice immunized alhydrogel F1-V partial preparations and controls with are provided in my notebook #3598 on pages 125-126. See Exhibit GA2.
9. The results dated **[redacted date which is before 13 March 1996]** for the challenge studies using *Yersinia pestis* strain C12, which is $F1^-$ strain, with mice immunized with alhydrogel F1-V partial preparations and controls with are provided in my notebook #3598 on pages 127-130. See Exhibit GA3.
10. On **[redacted date which is before 13 March 1996]**, I wrote in my notebook #3598 on page 132, that the data on page 131 of my notebook and the mouse challenge studies are the first direct evidence that the F1-V fusion (F1-V partial) can induce an immune response to both F1 and V protein. David G. Heath witnessed this page and the results to which it references. See Exhibit GA4.
11. Exhibit AF3 (GA5) is an excerpt of my notebook #3598.
12. On **[redacted date which is before 13 March 1996]**, I gave David G. Heath the protocol for formulating the F1-V whole vaccine preparations for mouse challenge assays. See Exhibit

DH16 (GA6).

13. In my laboratory notebook, I usually referred to the F1-V whole as "F1-WV".

14. Before about **[redacted date which is before 13 March 1996]**, I obtained alhydrogel F1-V whole preparations from David G. Heath and began conducting the mouse challenge studies. See Exhibit GA7.

15. The results of the challenge studies dated **[redacted date which is before 13 March 1996]** using *Yersinia pestis* strain C12 or CO92 with mice immunized with alhydrogel F1-V partial preparations and controls are provided in my notebook #3739 on pages 60-63. The results show that F1-V whole confer a protective immune response against both F^+ and F^- *Yersinia pestis* strains. See Exhibit GA8.

16. From about **[redacted date which is before 13 March 1996]** to 27 August 1996, I conducted further experiments to determine the efficacy of the F1-V fusion proteins and to determine whether any refinements could be made, such as the following:

    a. On **[redacted date which is before 13 March 1996]**, I conducted a mouse challenge study examining the long term efficacy of F1-V whole which is documented in my notebook #3739, page 75. See Exhibit GA9. The results dated **[redacted date which is before 13 March 1996]** are provided in my notebook #3739, page 85. See Exhibit GA10.

    b. On **[redacted date which is before 13 March 1996]**, I conducted a mouse challenge study examining the range of Al concentration which maintains an adequate adjuvant response which is documented in my notebook #3739, page 88. See Exhibit GA11. The alhydrogel F1-V whole preparations were obtained from David G. Heath. I copied the notebook pages from David G. Heath's notebook and inserted in my notebook. See Exhibit GA12. The results dated **[redacted date which is before 13 March 1996]** are found in my notebook #3739, pages 104-107. See Exhibit GA13.

    c. Exhibit GA14 shows experimental data from my notebook #3739 which evidence that from **[redacted date which is before 13 March 1996]** to 23 February 1996, I conducted various mouse challenge studies with F1-V whole.

    d. On 3 April 1996, I obtained the results for ELISA assays of serum obtained from mice immunized with F1-V whole to determine if the sera still contained antibodies against F1 antigen and V antigen as evidenced in my notebook #3739, page 122. See Exhibit GA15.

    e. On about 15 May 1996, I conducted a study with mice vaccinated with different amounts of F1-V whole protein. The mice were challenged subcutaneously and by aerosol with Y. pestis, C092 or C12. See Exhibit GA16.

    f. On about 28 June 1996, I conducted a study with mice vaccinated with either F1-V whole or a mixture of F1 + V or Plague USP vaccine. The mice were challenged by aerosol with Y. pestis C092. See pages 134 and 137 of my notebook #3739, Exhibit GA17.

    g. On about 5 July 1996, I conducted a study with mice vaccinated with either F1-V whole or F1 + V or Plague USP vaccine. The mice were challenged

subcutaneously and by aerosol with Y. pestis, C092 or C12. See pages 135-136 of my notebook #3739, Exhibit GA18.

17. For all the challenge studies referenced herein, I obtained most of the *Yersinia pestis* strains C12 and CO92 from Susan L. Welkos.

18. On 15 February 1996, I presented the work summarized in David G. Heath's Abstract 17. See Exhibit DH19 (GA19).

19. I left the Army Plague Vaccine Group on 26 February 1998 when I retired from the U.S. Army.

20. I have reviewed and analyzed the Titball patent and the three priority documents, UK 9505059, UK 9518946, and UK 9524825, and PCT/GB96/00571.

21. It is my opinion that prior to 13 March 1996, the filing date of PCT/GB96/00571, the inventors of the Titball patent had not conceived and/or reduced to practice a plague vaccine comprising purified F1 antigen fused to all or part of V antigen as nowhere do UK 9505059, UK 9518946, and UK 9524825 disclose isolating or purifying a protein comprising F1 antigen fused to all or part of V antigen from the host cell and other cellular components and/or administering a purified protein comprising F1 antigen fused to all or part of V antigen to a subject.

    a. In fact, UK 9518946 is the first disclosure indicating a genetic vaccine or how a host organism may be transfected with DNA for F1 antigen and V antigen to result in a live vaccine, i.e. an attenuated host organism (such as Salmonella) which produces the antigen when administered to a subject.

    b. As described in UK 9518946, the genetic vaccine or the live vaccine is administered to a subject such that the protein/antigen of interest is then produced in the subject.

    c. UK 9518946 does not describe isolating the protein/antigen of interest from the host organism and purifying the protein/antigen of interest from other cellular components prior to administration to a subject.

    d. The genetic vaccine or live vaccine described in UK 9518946 is not a purified protein comprising F1 antigen fused to all or part of V antigen which is isolated and purified from cells and other cellular components as claimed in the above-referenced application.

22. I have reviewed and analyzed the experiments and data of the Army Plague Vaccine Group and it is my opinion that the Army Plague Vaccine Group:

    a. Conceived of a fusion protein comprising F1 antigen fused to part of V by at least **[redacted date which is before 13 March 1996]**.

    b. Conceived of a fusion protein comprising F1 antigen fused to all of V by at least **[redacted date which is before 13 March 1996]**.

    c. Conceived of and reduced to practice a purified fusion protein comprising F1 antigen fused to part of V by at least **[redacted date which is before 13 March 1996]**.

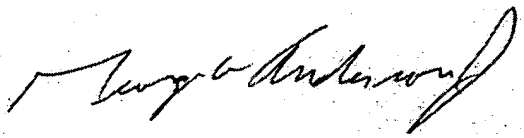
    d. Conceived of and reduced to practice a purified fusion protein comprising F1

antigen fused to all of V by at least **[redacted date which is before 13 March 1996]**.

e. Conceived of and reduced to practice a vaccine against plague comprising a purified fusion protein comprising F1 antigen fused to part of V by at least **[redacted date which is before 13 March 1996]**.

f. Conceived of and reduced to practice a vaccine against plague comprising a purified fusion protein comprising F1 antigen fused to all of V by at least **[redacted date which is before 13 March 1996]**.

23. I declare that all statements made herein of my own knowledge are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.



Date: 14 March 2007

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CURRICULUM VITAE

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PROFESSIONAL EXPERIENCE:

2004-Present	Senior Principal Engineer. Scientific responsibilities for the Operations and Sustainment of a Defense Threat Reduction sponsored multi-country epidemiological surveillance system and collaborative biological research program in the former Soviet Union.
2003-2004	Principal Advisor. Midwest Research Institute. General scientific guidance to the company and responsibilities for integrating the capabilities of various company divisions in projects. Continue participation in DTRA projects in the former Soviet Union (Kazakhstan, Uzbekistan and Republic of Georgia). Senior Program Manager. Department at Southern Research Institute was purchased by Midwest Research Institute. I continued program management of the work in the former Soviet Union until promoted to Principal Advisor.
1998-2003	Director, Medical Countermeasures Department. Southern Research Institute. Responsible for the management, direction, control and review of the departmental research and development programs. Established a Biological Safety Level-3 containment facility for vaccine potency testing of Department of Defense (DoD) Investigational New Product (IND) vaccines (VEE, WEE, EEE, Q fever, Tularemia) in Frederick, MD. Department provided the DoD with expertise in biological defense, biosecurity, biotechnology and

biosafety for DARPA and DTRA projects at former biological weapons facilities in the former Soviet Union (FSU). Some of the projects support Non-proliferation efforts. Projects in the FSU include on-site observations of laboratory work in BSL-2, -3 and BSL-4 containment laboratories. Worked as a consultant to the Department of the Army for the anthrax vaccine production facility in the United States. Conducted audits in BSL-3 laboratories. Immunized with most licensed and IND bio-defense vaccines. Biosafety consultant to U.S. laboratories. Technical spokes person on biosafety for dismantlement of a pilot production facility for biological warfare agents in the United States.

1998-1998 Principal Scientist, SRS Technologies. Perform and manage scientific and technical tasks requiring the assessment of chemical (CW) or biological warfare (BW) programs/capabilities of foreign countries for terrorist groups and the development of recommended measures to curb the proliferation of biological weapons and technologies.

1993-1998 Chief, Pathogenesis and Immunology Branch, U.S. Army Medical Research Institute of Infectious diseases (USAMRIID). Branch Chief responsible for directing research programs directed toward development and production of prophylactic and therapeutic modalities against bacterial diseases of potential biological warfare significance (e.g., anthrax, plague, Q fever, tularemia, glanders). Special project officer for multi-million dollar Good Laboratory Practices project which included facility upgrades, Responsible for the GLP BSL-3 laboratory setup, and project manager for multi-year preclinical project for a supplement to the anthrax vaccine license. Research associate on the clinical protocol for a supplement to the current anthrax vaccine license. Manage technical aspects of a contract for cGMP production of cell banks and recombinant *Bacillus anthracis* PA protein as diagnostic or vaccine component. Involved with nonproliferation activities with former Soviet weapons scientist. First US military officer invited into the BSL-3 containment laboratories at the State Research Center of Applied Microbiology; Obolensk, Russia.

1990-1993 Medical R&D Officer, Science and Technology Center-Europe, Frankfurt, Germany - Responsible for finding biotechnologies, products, and collaboration in Europe, the Middle East, Africa, and the former Soviet Union, which could shorten or negate the need for the R&D cycle in Defense Department laboratories. This work involved extensive travel to scientific conference and Institutes in these geographical areas and technical report writing.

1987-1990	Research Immunologist, U.S. Army Medical Research Institute of Infectious Diseases. Investigations included development of a congenic strain of rats, vaccine efficacy trials against an aerosol exposure, and pathogenesis studies on Rift Valley fever virus. These studies were carried out in a Biosafety Level 3 laboratory.
1983-1987	Graduate student at the Johns Hopkins University, while on active duty, US Army.
1977-1983	Research Scientist, U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID), Fort Detrick, Frederick, Maryland - Was responsible for developing and investigating genetically define animal models for exotic viral and rickettsial diseases.

ACADEMIC APPOINTMENT: Associate Professor, Center for Disaster Preparedness, School of Medicine, University of Alabama

FIELD STUDIES:

1988	Member of a team of two who conducted an epidemiological sero-survey for phleboviral infections at the MRC Laboratory, The Gambia, and at the Institute Pasteur, Senegal, West Africa.

MILITARY SERVICE:

1977-1998	Retirement rank: LTC, U.S. Regular Army Commission, MSC Army Management Staff College, graduate Command and General Staff College, graduate

EDUCATION:

Ph.D., 1988	The Johns Hopkins University, Baltimore, Maryland, Viral Immunology. Dissertation: *Viral and Host Determinants of Resistance to Rift Valley Fever in a Rat Model*
M.S., 1977	Florida Institute of Technology, Melbourne, Florida, Biology.
B.S., 1975	Florida Institute of Technology, Melbourne, Florida, Biology.

TEACHING EXPERIENCE:

1996	Mentor, Department of Defense, Science & Engineering Apprentice Program

1989-1990	Served as a thesis committee member for a master's level candidate at Hood College, Frederick, Maryland. Supervised the candidate's research.
1988-1989	Sponsored a Korean ophthalmologist for the 1988-1989 ROK/US Scientist/Engineer Exchange Program in my laboratory to develop a model to study Rift Valley fever ocular sequelae.
1975-1977	Teaching assistantship at Florida Institute of Technology with primary teaching responsibilities for microbiology and biochemistry laboratories.

MEMBERSHIP IN ACADEMIC AND PROFESSIONAL SOCIETIES:

Membership: American Society for Microbiology
Sigma XI Scientific Research Society

HONORS AND AWARDS:

Who's Who Among Students in American Universities – 1974, 1975
Four-year Army ROTC Scholarship
Four-year U.S. Army Long Term Health, Education and Training Program (Ph.D. scholarship to the Johns Hopkins University)
Blue Key National Honor Fraternity
Sigma XI Scientific Research Society
Scouting: Highest rank – Eagle, Highest honor – Order of the Arrow, Vigil Member
U.S. Army Army Commendation Medal
Meritorious Service Award w/2 Oak leaf clusters
Legion of Merit
Distinguished Professional Achievement Award, Florida Institute of Technology 2002
Letter of appreciation from Joint Program Office for Biological Defense for assistance to BioPort Corporation in obtaining FDA approval for Biological License Agreement for anthrax vaccine

PATENTS:

U.S. Patent Number 5,320,069, "Small Animal Restraint Device"
Patent Pending Recombinant F1-V Plague Vaccine, filing #08/699,716 18 Dec 96

CONTINUING EDUCATION:

The Regulatory Process and Good Clinical Practices, Technology Management Integration, Inc., 1994

Regulatory Issues in Biotechnology, Univ. MD, 1995
Good Manufacturing Processes for Bioprocesses, Univ. MD, 1996
Quality Control and Quality Assurance of Biotechnology Products, Univ. MD, 1996
Assay Validation, PDA, 1996
Intro.to GLPs and Auditing, International Center for Health & Environmental Education, 1996
Writing and Evaluating Standard Operating Procedures for the Regulatory Environment, International Quality Training, 1996
Intro. FDA Good Laboratory Practices & Documentation Principles, International Quality Training, 1996
Good Laboratory Practices Regulations for Study Directors, International Quality Training, 1996
Fundamentals & Concepts of Calibration & Metrology, PDA, 1996
Biotechnology GMP Facility Design, Construction and Validation, Univ. MD, 1997
Advances in Filtration and Bioseparation Technologies, Pall Ultrafine Filtration Company, Columbia, MD, 1997
Validation of Biotechnology Processes and Systems, Univ. MD, 1997
Fermentation Microbiology, American Type Culture Collection Workshop, Rockville, MD, 1997
Fundamentals of D, F and Z Values, PDA, 1997
Basic Principles in Preparation of Sterile Dosage Forms, PDA, 1997
Parenteral Packaging: Rubber, Glass, Plastic and Metal Seals, PDA, 1997
Regulatory Compliance Training, Southern Research Institute, 1998
ISO 9001/Quality system Introductory Training, Southern Research Institute, 1998
Introductory to Earned Value Seminar, Dynport, LLC Professional Development, 1998
Positive Pressure Pneumatic BSL-4 Suite Training, State Research Center of Virology and Biotechnology, "Vector", Russia 2000
Introduction To Aerosol Mechanics I & II, AAAR, 2000
USA-Russia Workshop on International Research Ethics; Institutional Review Boards and Laboratory Animal Welfare, 2002
Introduction to Laboratory Ventilation and Design, American Biological Safety Association, 2002
Plant Biosafety, American Biological Safety Association, 2002
Bechtel Safety Leadership Workshop, Bechtel National Inc, 2006
The Transport of diagnostic & Infectious Samples, American Biological Safety Association, 2006
Biohazard Risk Assessment, American Biological Safety Association, 2006

Certifications:

Transport of diagnostic & Infectious Substances by Air (per ICAO Technical Instructions & IATA DGR) valid until 15 Oct 2008

REVIEWER/CONSULTANT:

U.S. Army In-house laboratory independent Research (ILIR) proposals, 1993-1998
U.S. Army Broad Agency Announcement proposals, 1993-1998
Experts Contact for database to assist Biological Arms Control Treaty Office (BACTO), 1997-1998
Nonproliferation Programs IntraAgency Roundtable, 1997-1998
U.S.-Uzbek Collaborative Biotechnology Grants Program, 2000
Consultant for DoD at BioPort Corporation (anthrax vaccine production facility), 2000-2002

PROFESSIONAL PUBLICATIONS:

1. **Anderson, G.W., Jr.**, and J.V. Osterman. 1980. Host defenses in experimental Rickettsialpox: Genetics of natural resistance to infection. Infect. Immun. 28: 132-136.

2. **Anderson, G.W., Jr.**, and J.V. Osterman. 1980. Host defenses in Rickettsialpox: Resistance of C3H mouse sublines. Acta. Virol. 24: 294-296.

3. Peters, C.J., and **G.W. Anderson, Jr.** 1981. Pathogenesis of Rift Valley fever, pp. 21-41. In Contributions to Epidemiology and Statistics, vol. 3. (N. Goldblum, T.A. Swartz, and M.A. Kingberg, eds.). S. Karger, Basel.

4. **Anderson, G.W., Jr.**, T.W. Slone, Jr., and C.J. Peters. 1987. Pathogenesis of Rift Valley fever virus (RVFV) in inbred rats. Microb. Pathogen. 2: 283-293.

5. **Anderson, G.W., Jr.**, and J.F. Smith 1987. Immunoelectron microscopy of Rift Valley fever viral morphogenesis in primary rat hepatocytes. Virology. 161: 91-100.

6. **Anderson, G.W., Jr.**, and C.J. Peters. 1988. Viral determinants of virulence for Rift Valley fever (RVF) in rats. Microb. Pathogen. 5: 241-250.

7. **Anderson, G.W., Jr.**, T.W. Slone, Jr., and C.J. Peters. 1988. The gerbil, *Meriones unguiculatus*. A model for Rift Valley fever viral encephalitis. Archives Virology 102: 187-196.

8. **Anderson, G.W., Jr.**, J.-F. Saluzzo, T.G. Ksiazek, J.F. Smith, W. Ennis, D. Thureen, C.J. Peters, and J.P. Digoutte. 1989. Comparison of in vitro and in vivo systems for propagation of Rift Valley fever virus from clinical specimens. Res. Virol. 140:129-138.

9. Saluzzo, J.F., **G.W. Anderson, Jr.**, L.A. Hodgson, J.P. Digoutte, and J.F. Smith. 1989. Antigenic and biological properties of Rift Valley fever virus isolated during the 1987 Mauritanian epidemic. Res. Virol. 140:155-164.

10. Peters, C.J., C.-T. Liu, **G.W. Anderson, Jr.**, J.C. Morrill, and P.B. Jahrling. 1989. Pathogenesis of viral hemorrhagic fevers: Rift Valley fever and Lassa fever contrasted. Rev. Infect. Dis. 11 (Suppl. 4): 5743-5749.

11. Saluzzo, J.F., **G.W. Anderson, Jr.**, J.F. Smith, D. Fontenille, and P. Coulanges. 1989. Biological and antigenic relationship between Rift Valley fever virus strains isolated in Egypt and Madagascar. Trans. R. Soc. Trop. Med. Hyg. 83:701.

12. Solow, R., K. Mereish, **G.W. Anderson, Jr.**, and J. Hewetson. 1990, Effect of microcystin-LR on cultured rat endothelial cells. Med. Sci. Res. 18:241-244.

13. **Anderson, G.W., Jr.**, M.V. Slayter, W. Hall, and C.J. Peters. 1990. Pathogenesis of a phleboviral infection (Punta Toro virus) in Golden Syrian hamsters. Arch. Virol. 114: 203-212.

14. **Anderson, G.W., Jr.**, J.0. Lee, A.0. Anderson, N. Powell, J.A. Mangiafico, and G. Meadors. 1991. Efficacy of a Rift Valley fever virus vaccine against an aerosol infection in rats. Vaccine. 9: 710-714.

15. **Anderson, G.W., Jr.**, J.A. Rosebrock, A.J. Johnson, G.B. Jennings, and C.J. Peters. 1991. Infection of inbred rats with Rift Valley Fever virus: Development of a congenic resistant strain and observations on age-dependence of resistance. Am. J. Trop. Med. Hyg. 44(5): 475-480.

16. **Anderson, G.W., Jr.**, W.B. Lawrence, J-O Lee, and M. Young. 1991. A restraint for ophthalmic examination of unanesthetized rats. Note. Laboratory Animal Science. 41(3): 288-290.

17. Friedlander, A.M., S.L. Welkos, P.L. Worsham, G.P. Andrews, D.G. Heath, **G.W. Anderson, Jr.**, M.L.M. Pitt, J. Estep, and K. Davis. 1995. Relationship between virulence and Immunity as revealed in recent studies of the F1 capsule of Yersinia pestis. Clinical Infectious Diseases. 21(Suppl 2):S178-81.

18. Andrews, G.P., D.G. Heath, **G.W. Anderson, Jr.**, S.L. Welkos, and A.M. Friedlander. 1996. Fraction 1 capsular antigen (F1) purification from *Yersinia pestis* CO92 and an *Escherichia coli* recombinant strain and efficacy against lethal plague challenge. Infect. Immun. 64:2180-2187.

19. **Anderson, G.W. Jr.**, S.E.C. Leary, E.D. Williamson, R.W. Titball, S.L. Welkos, P.L. Worsham, and A.M. Friedlander. 1996. Recombinant V antigen protects mice against pneumonic and bubonic plague caused by F1-capsule-positive and -negative strains of *Yersinia pestis*. Infect. Immun. 64:4580-4585.

20. **Anderson, G.W. Jr.**, P.L. Worsham, C.R. Bolt, G.P. Andrews, S.L. Welkos, A.M. Friedlander, and J.P. Burans. 1997. Protection of mice from fatal bubonic and pneumonic plague by passive immunization with monoclonal antibodies against the F1 protein of *Yersinia pestis* . Am. J. Trop. Med. Hyg. 64:4580-4585.

21. Heath, D.G., **G.W. Anderson, Jr.**, S.L. Welkos, A.M. Friedlander, and J.M. Mauro. 1997. A recombinant capsular F1-V antigen fusion protein vaccine protects against experimental bubonic and pneumonic plague. *in* Vaccines 97. Cold Spring Harbor Laboratory Press, pp 197-200.

22. Pullen, J.K., **G.W. Anderson, Jr.**, S.L. Welkos, and A.M. Friedlander. 1998. Analysis of the *Yersinia pestis* V protein for the presence of linear antibody epitopes. Infect. Immun. 66:521-527.

23. **Anderson, G.W. Jr.**, D.G. Heath, C.R. Bolt, S.L. Welkos, and A.M. Friedlander. Short- and long-term efficacy of single-dose subunit vaccines against *Yersinia pestis* in mice. Am. J. Trop. Med. Hyg., 58(6): 793-799.

24. Ivins, B.E., M.L.M. Pitt, P.F. Fellows, J.W. Farchaus, G.E. Benner, D.M. Waag, S.F. Little, **G.W. Anderson, Jr.**, P.H. Gibbs, and A.M. Friedlander. 1998. Comparative efficacy of experimental anthrax vaccine candidates against inhalation anthrax in rhesus macaques. Vaccine, 16(11/12): 1141-1148.

25. Heath, D.G., **G.W. Anderson, Jr.**, J.M. Mauro, S.L. Welkos, G.P. Andrews, J. Adamovicz, and A.M. Friedlander. 1998. Protection against experimental bubonic and pneumonic plague by a recombinant capsular F1-V antigen fusion protein vaccine. Vaccine, 16(11/12): 1131-1137.

26. Andrews, G.P., S.T. Strachan, G.E. Benner, A.K. Sample, J.J. Adamovicz, **G.W. Anderson, Jr.**, S.L. Welkos, A.M. Friedlander. 1999. Protective efficacy of recombinant *Yersinia* outer proteins (Yops) against bubonic plague caused by encapsulated and non-encapsulated *Yersinia pestis*. Infect. Immun., 67(3): 1533-1537.

27. Lawrence W.B., **G.W. Anderson, Jr.**, J.0. Lee, and W.C. Hall. Ocular sequelae associated with Rift Valley fever virus (RVFV) infection in inbred rats (submitted).

PUBLISHED ABSTRACTS:

1. Rosebrock, J.A., **G.W. Anderson, Jr.**, H. Schellekens, and C.J. Peters. 1983. Differential interferon sensitivities of lethal and non-lethal strains of Rift Valley fever virus (RVFV) in vitro. In vitro 19: 286-287.

2. **Anderson, G.W., Jr.**, M.V. Slayter, and C.J. Peters. 1988. Pathogenesis of a phleboviral infection (Punto Toro virus) in golden Syrian hamsters. Virus Supplement 2: 40.

3. Ribas, J.L., M.D. Kanzer, **G.W. Anderson, Jr.**, J. Sesterhenn, and C.J. Peters. 1989. Rift Valley fever viral encephalitis in the gerbil. J. Neuropathol. Exp. Neurol. 48: 315.

4. Ribas, J.L., M.D. Kanzer, **G.W. Anderson, Jr.**, E. Perez-Rosario, and C.J. Peters. 1990. Rift Valley fever viral encephalitis in the gerbil: Ultrastructural and immunocytochemical correlation. J. Neuropath. Exp. Neurol. 49:348(A).

Other publications:

Technical Report for Alternate Air Collection Media, SRS Technologies, TR98-156

PRESENTATIONS:

1. **Anderson, G.W., Jr.**, and J.V. Osterman. Susceptibility of mouse strains to *Rickettsia akari*. Presented at the 79th Annual Meeting of the American Society of Microbiologists, Los Angeles, California, 1979.

2. **Anderson, G.W., Jr.**, and J.V. Osterman. Susceptibility of mice to *Rickettsia akari*. Presented at the 1st Annual Rickettsiology Conference. Port Deposit, Maryland, 1979.

3. **Anderson, G.W., Jr.**, and C.J. Peters. Effect of immunosuppression on genetically resistant LEWIS/Mai rats to Rift Valley fever virus. Presented at the Maryland-D.C. Branch of the American Society for Microbiology Meeting, Fort Detrick, Frederick, Maryland, January 1981.

4. **Anderson, G.W., Jr.**, C.J. Peters, and T.W. Slone. Pathogenesis of Rift Valley fever virus in inbred rats. Presented at the 81st Annual Meeting of the American Society for Microbiology, Dallas, Texas, March 1981. Abstracts of the Meeting, D244.

5. Peters, C.J., and **G.W. Anderson, Jr.** Pathogenesis of Rift Valley fever and other Phlebovirus infections. Presented at the 5th International Congress of Virology, Strasbourg, France, August 1981.

6. Peters, C.J., and **G.W. Anderson, Jr.** Resistance to Phleboviruses. Presented at the U.S.-Japan Cooperative Medical Science Program, Bethesda, Maryland, 9 November 1981.

7. Peters, C.J., and **G.W. Anderson, Jr.** Pathogenesis of Phlebovirus infections. Presented at the 30th Annual Meeting of the American Society of Tropical Medicine and Hygiene, San Juan, Puerto Rico, November 1981.

8. **Anderson, G.W., Jr.**, and C.J. Peters. Role of humoral immunity in Rift Valley fever infection. Presented at the 49th Conjoint Meeting on Infectious Diseases, Ontario, Canada, 25 November 1981.

9. **Anderson, G.W., Jr.**, J.A. Rosebrock, A.J. Johnson, and C.J. Peters. Age- and dose-dependent resistance of rats to Rift Valley fever virus. Presented at the 82nd Annual Meeting of the American Society for Microbiology, Atlanta, Georgia, March 1982.

10. **Anderson, G.W., Jr.**, T,W. Slone, Jr., and C.J. Peters. A model for the encephalitic form of Rift Valley fever. Presented at the 31st Annual Meeting of the American Society of Tropical Medicine and Hygiene, Cleveland, Ohio, 1982.

11. Peters, C.J., H. Schellekens, J.A. Rosebrock, and **G.W. Anderson, Jr.** Genes, macrophages, and resistance to Rift Valley fever in the rat. Presented at the First Annual Meeting of the American Society for Virology, Ithaca, New York, 1982.

12. Peters, C.J., H. Schellenkens, J.A. Rosebrock, and **G.W. Anderson, Jr.** Genetic resistance to Rift Valley fever virus: Role of macrophages and interferon. Fourth International Conference on Comparative Virology, Banff, Canada, October 1982.

13. **Anderson, G.W., Jr.**, and J.F. Smith. Rift Valley fever virus (RVFV) maturation at the plasma membrane of rat hepatocytes as revealed by immunoelectron microscopy. Presented at the 35th Annual Meeting of the American Society of Tropical Medicine and Hygiene, Denver, Colorado; 8-11 December 1986.

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File: F1-V fusion last update REDACTED
Protocol: B94-02
F1-V fusion protein immunization and challenge

Investigators: CPT Heath, Dr. Welkos, LTC Anderson, COL Friedlander

Background. CPT David Heath has produced and purified a recombinant F1-V fusion protein. The protein is positive by Western blot to F1 and V. Antigen dose will be based on 10 µg F1/dose + the amount of V which is fused to it. Subcutaneous injection at nape of neck.

V-antigen used in this experiment is from Mauro. Details of the V-antigen can be obtained from CPT Heath's notebook #_____ page _____.

Purpose: Immunize and challenge mice to check on immunogenicity and protection against the CO92 strain of Y. pestis by sc and aerosol challenge, **50 LD_{50}**.

EcF1c will be endotoxin free, same F1 as used in the active immunization experiment.

Alhydrogel, 1.3%, from SuperFos. Batch # 2043, Expiration date None, _____µg of AL/dose

Immunization Groups: 10 Swiss Webster female mice per group from Harlan Sprague Dawley

Groups **1-9**, the amount of F1 will be held constant.

		Strain	# Mice
Subcutaneous challenge			
Group 1	Alhydrogel alone, days 0, 30, sc	CO92	10
Group 2	alhydrogel + 10 µg F1, days 0, 30, sc	CO92	10
Group 3	alhydrogel + 10 µg F1 urea treated, days 0, 30, sc	CO92	10
Group 4	Alhydrogel + 18.5 µg F1-V fusion protein days 0, 30, sc	CO92	10
Aerosol challenge			
Group 5	Alhydrogel alone, days 0, 30, sc	CO92	10
Group 6	alhydrogel + 10 µg F1, days 0, 30, sc	CO92	10
Group 7	alhydrogel + 10 µg F1 urea treated, days 0, 30, sc	CO92	10
Group 8	Alhydrogel + 18.5 µg F1-V fusion protein, days 0, 30, sc	CO92	10
Group 9	Alhydrogel + 37.0 µg F1-V fusion protein days 0, 30, sc	CO92	10
		Total	90

1F622A6174/F-V-000
1F633B5D66/F-V-001
1F666A563B/F-V-002
1F684B1B13/F-V-003
1F73143D1D/F-V-004
1F6336192F/F-V-005
1F617A0402/F-V-006
1F6373612A/F-V-007
1F642A0E45/F-V-008
1F64667324/F-V-009
1F610F757C/F-V-010
1F625B356F/F-V-011
1F62584463/F-V-012
2007432B6B/F-V-013
1F620D6B07/F-V-014
1F65793A49/F-V-015

REDACTED

1F61041D5F/F-V-016
1F59570130/F-V-017
1F62776325/F-V-018
1F6E2E3015/F-V-019
1F60055D1F/F-V-020
1F66337F49/F-V-021
1F6E25735B/F-V-022
1F647B4141/F-V-023
1F6835251F/F-V-024
1F767F7A72/F-V-025, Not responding replaced with 1F617C1371
1F62530428/F-V-026
1F63717815/F-V-027
1F65122842/F-V-028
1F666F1F6D/F-V-029
1F56376371/F-V-030
1F66655A3C/F-V-031
1F663A6859/F-V-032
1F64163037/F-V-033
1F72107B64/F-V-034
1F63467B3D/F-V-035
1F645B1111/F-V-036
1F61524668/F-V-037
1F6132321C/F-V-038
1F655A0E14/F-V-039
1F661E3726/F-V-040, Not responding, replaced with 1F6134004C
1F656C5937/F-V-041
1F61135D10/F-V-042
1F6655574F/F-V-043
1F664B210F/F-V-044
7F7B0A2C5B/F-V-045
1F63737516/F-V-046
1F66240C4B/F-V-047
1F5A6F0C0C/F-V-048
1F65354106/F-V-049
1F76011A50/F-V-050
1F653D7946/F-V-051
1F624D6A48/F-V-052
1F64030B6F/F-V-053
1F6E295D6D/F-V-054
1F64303B12/F-V-055
1F635D326F/F-V-056
1F6726084C/F-V-057
1F6317796E/F-V-058
1F64024437/F-V-059
1F66056C0A/F-V-060, died from anesthesia on17FEB95 during bleeding
1F637E6917/F-V-061
1F64653F59/F-V-062
1F643F7846/F-V-063
1F6412204B/F-V-064
7F7D261230/F-V-065
1F6132735B/F-V-066
1F635C5D45/F-V-067
1F636A1103/F-V-068
1F5F542F7F/F-V-069

1F6729527F/F-V-070
1F63280353/F-V-071
1F600B6D09/F-V-072
1F77037275/F-V-073
1F615E071B/F-V-074
1F646F0D01/F-V-075
1F66415A60/F-V-076
1F65566046/F-V-077
1F64495262/F-V-078
1F6467682E/F-V-079
200905054D/F-V-080
1F636F2F60/F-V-081
1F650E541A/F-V-082
1F637B3E45/F-V-083
1F673C5668/F-V-084
1F647E2E51/F-V-085
1F663B0937/F-V-086
1F62111658/F-V-087
1F6320124C/F-V-088
1F635A1F05/F-V-089

CFA from Sigma Cat#F-5881 Lot#80H8808, 10 ml/bottle
IFA from Sigma Cat#F-5506 Lot#80H8812, 10 ml/bottle

Groups **10-23** will be started one week later. The amount of V will be held constant, though the V part of the fusion protein is only about half the size of the native V.

		Strain	# Mice
Subcutaneous challenge			
Group 10	Alhydrogel alone, days 0, 30, sc	C12	10
Group 11	Alhydrogel days + 27 µg F1-V fusion protein day 0, 30, sc	C12	10
Group 12	CFA + 10 µg V, days 0, IFA 30, ip	C12	10
Group 13	CFA + 10 µg V urea treated, days 0, IFA 30, ip	C12	10
Group 14	CFA + 27 µg F1-V fusion protein days 0, IFA 30, ip	C12	10
Group 15	CFA + 54 µg F1-V fusion protein days 0, IFA 30, ip	C12	10
Group 16	CFA day 0, IFA day 30 alone, ip	C12	10
Aerosol challenge			
Group 17	Alhydrogel alone, days 0, 30, sc	C12	10
Group 18	Alhydrogel + 27 µg F1-V fusion protein, days 0, 30, sc	C12	10
Group 19	CFA + 10 µg V, days 0, IFA 30, ip	C12	10
Group 20	CFA + 10 µg V urea treated, days 0, IFA30, ip	C12	10
Group 21	CFA + 27 µg F1-V fusion protein day 0, IFA 30, ip	C12	10
Group 22	CFA + 54 µg F1-V fusion protein day 0, IFA 30, ip	C12	10
Group 23	CFA day 0, IFA day 30 alone, ip	C12	10
		Total	140
Group 24	CFA + 27 µg F1-V fusion protein day 0, IFA 30, ip --antibody response Measure titer at 14, 27,57		10

Chip # for Groups 10-24, Group 10 has some mice doubled
1F646A0D06/F-V-090
7F7B107C58/F-V-091 or 1F63125319, DOUBLE CHIPPED
1F6E345D62/F-V-092 OR 1F64791E66, DOUBLE CHIPPED

1F64141752/F-V-093 OR 1F61116E01, DOUBLE CHIPPED
1F65020D6D/F-V-094 OR 9F7D25797E, DOUBLE CHIPPED
1F62032B51/F-V-095 OR 1F650627AF, DOUBLE CHIPPED
1F635D4B56/F-V-096 OR 7F7D243700, DOUBLE CHIPPED
7F7B06623C/F-V-097 OR 7F7D17224D, DOUBLE CHIPPED
7F7D23252D/F-V-098
1F630A7004/F-V-099
1F6458061F/F1-VB-001
1F66294012/F1-VB-002
1F65323119/F1-VB-003
1F68597E22/F1-VB-004
1F663B023E/F1-VB-005
1F64742F5A/F1-VB-006
1F62713757/F1-VB-007
1F664A1B16/F1-VB-008
1F624C3D76/F1-VB-009
1F60466754/F1-VB-010
1F650D343B/F1-VB-011
1F640F0668/F1-VB-012
1F684A6D42/F1-VB-013
1F627B4440/F1-VB-014
1F66362421/F1-VB-015
1F64121C4F/F1-VB-016
1F647A4340/F1-VB-017
1F757C7977/F1-VB-018
1F65625644/F1-VB-019
1F65662E68/F1-VB-020
1F655A455D/F1-VB-021, found dead, 13JAN95, cause unknown
1F63624B51/F1-VB-022
1F65487440/F1-VB-023
1F61185F09/F1-VB-024
1F63296273/F1-VB-025
1F6626094C/F1-VB-026
1F622A1C39/F1-VB-027
1F6122312D/F1-VB-028
1F6329775E/F1-VB-029
1F64652276/F1-VB-030
1F73061751/F1-VB-031
1F63672C6B/F1-VB-032
1F68406158/F1-VB-033
1F6359061F/F1-VB-034
1F653E2C12/F1-VB-035
1F60524B64/F1-VB-036
1F67274A09/F1-VB-037
200F13231B/F1-VB-038
1F620B7B79/F1-VB-039
1F66121653/F1-VB-040
1F66247661/F1-VB-041
1F652F0D40/F1-VB-042
1F63103B33/F1-VB-043
1F673D0C31/F1-VB-044
1F62757218/F1-VB-045
1F63401727/F1-VB-046
1F665F5D3F/F1-VB-047

1F66585F44/F1-VB-048
1F61195611/F1-VB-049
200737366C/F1-VB-050
1F614E2012/F1-VB-051
1F64297C58/F1-VB-052
1F75463175/F1-VB-053
1F626E157C/F1-VB-054
1F650F5419/F1-VB-055
1F64075C1A/F1-VB-056
1F756A3151/F1-VB-057
1F6121124D/F1-VB-058
1F655E405E/F1-VB-059
1F65233C1D/F1-VB-060
1F683C0B32/F1-VB-061
1F615F021F/F1-VB-062
1F71772257/F1-VB-063
1F644F713D/F1-VB-064
1F67412415/F1-VB-065
1F66606C2F/F1-VB-066
1F77087969/F1-VB-067
1F637D1F62/F1-VB-068
1F61172742/F1-VB-069
1F64511E0E/F1-VB-070
1F5756496B/F1-VB-071
1F681C4617/F1-VB-072
1F68571012/F1-VB-073
1F682F6763/F1-VB-074
1F673E4577/F1-VB-075
1F62681205/F1-VB-076
1F655F1E7F/F1-VB-077
1F65523377/F1-VB-078
1F61284810/F1-VB-079
1F6152307E/F1-VB-080
1F68382A17/F1-VB-081
1F686B1876/F1-VB-082
1F60106E03/F1-VB-083
1F63242D2D/F1-VB-084
1F637A7311/F1-VB-085
1F631F0D52/F1-VB-086
1F6378473F/F1-VB-087
1F63390C39/F1-VB-088
1F66517931/F1-VB-089
1F642C272A/F1-VB-090
1F654E703E/F1-VB-091
1F64134129/F1-VB-092
1F63355772/F1-VB-093
1F621A0263/F1-VB-094
1F64361A2D/F1-VB-095
1F61774A3F/F1-VB-096
1F65276075/F1-VB-097
1F646F0905/F1-VB-098
1F624C181B/F1-VB-099
1F684E3C6F/F1-VB-100
1F64034B2F/F1-VB-101

1F65083C38/F1-VB-102
1F65792E55/F1-VB-103
1F6012412E/F1-VB-104
1F68321F28/F1-VB-105
1F6E313909/F1-VB-106
1F614D6F44/F1-VB-107
1F62394402/F1-VB-108
1F627B5C28/F1-VB-109
1F64165A0D/F1-VB-110
1F630B4231/F1-VB-111
1F6629450D/F1-VB-112
1F5F630D12/F1-VB-113
1F657C5B25/F1-VB-114
1F6337596E/F1-VB-115
1F66297260/F1-VB-116
1F66524F5A/F1-VB-117
1F6829537D/F1-VB-118
1F64616E2E/F1-VB-119
1F61517936/F1-VB-120
1F65136504/F1-VB-121
1F66002A51/F1-VB-122
1F5F7D1075/F1-VB-123
1F65074134/F1-VB-124
1F71724836/F1-VB-125
1F656C4F41/F1-VB-126
1F60182148/F1-VB-127
1F6866573C/F1-VB-128
1F62780205/F1-VB-129
1F65080173/F1-VB-130

Schedule

Groups 1-9

22NOV94	Arrival of Swiss Webster mice, female 7-8 wks, Harlan Sprague Dawley in AA-3 (Barrier)
01DEC94	Chipped with BioMedic Data Systems transponders in AA-3
16DEC94	1st immunization, AA-3
13JAN95	2nd Immunization, AA-3, day 28
17FEB95	Bleed to determine prechallenge titers, AA-3, day 63, serum#1500-1589
24FEB95	Challenge by aerosol & sc routes, day 70
24MAR95	Terminal bleed, day 28 pi, titrate spleens, serum # 2403 - 2453 Tissue # 9378 - 9432

Groups 10-23

29NOV94	Arrival of Swiss Webster mice, female 7-8 wks, Harland Sprague Dawley in AA-3 (Barrier)
13DEC94	Chipped with BioMedic Data Systems transponders in AA-3 (SGT Zimmerman, Vet Med)
22DEC94	1st immunization, AA-3; Crow & Fritzgerald helped
20JAN95	2nd immunization, AA-3, day 29
24FEB95	Bleed to dermine prechallenge titers, AA-3, day 64, serum# 1628 - 1678
3MAR95	Challenge by aerosol & sc route, day 71
31MAR95	Terminal bleed, day 28 pi, titrate spleens, serum # 2754 - 2800 Tissue # 9433 - 9479

Group 24

22DEC94	1st immunization, AA-3
06JAN95	Bleed, day 14, AA-3, SERUM# 1220-1229
20JAN95	Bleed, day 27, AA-3, SERUM# 1339-1348
20JAN95	2nd immunization, day 28, AA-3
24FEB95	Bleed, day 63, AA-3, SERUM# 1768-1778 and bronchial lavage #

w page 123

Exhibit GA2

REDACTED

Project: Active Immunization F1-V with Alhydrogel

Notebook #: 3598

Inoculum: Yersinia pestis strain, CO92

Route: SC Dose: 63 ug

Swiss Webster Age: REDACTED at 7-8 wk Vendor: Harlan Sprague Dawley Sex: female

REDACTED

Day post infection	24	25	26	27	28	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	Comments/Chip #
Group	Cage#																														
Alh alone																															
GP1																															1F622A6174/F-V-000
1						D																									1F633B5D66/F-V-001
							D	D																							1F666A563B/F-V-002
								D																							1F684B1B13/F-V-003
				D																											1F73143D1D/F-V-004
					D	D																									1F6336192F/F-V-005
					D																										1F617A0402/F-V-006
						D	D																								1F6373612A/F-V-007
						D																									1F642A0E45/F-V-008
						D																									1F64667324/F-V-009
Alh+F1																															
10 µg																															1F610F757C/F-V-010
GP2																															1F625B356F/F-V-011
2																															1F62584463/F-V-012
																															2007432B6B/F-V-013
																															1F620D6B07/F-V-014
																															1F65793A49/F-V-015
																															1F61041D5F/F-V-016
																															1F59570130/F-V-017
																															1F62776325/F-V-018
																															1F6E2E3015/F-V-019
Alh+F1																															
0 µg																															1F60055D1F/F-V-020
urea																															1F66337F49/F-V-021
GP3																															1F6E25735B/F-V-022
3																															1F647B4141/F-V-023
																															1F6835251F/F-V-024
																															1F617C1371/F-V-025
																															1F62530428/F-V-026
																															1F63717815/F-V-027
																															1F65122842/F-V-028
																															1F666F1F6D/F-V-029
Alh+F1-V																															
8.5 µg																															1F56376371/F-V-030
GP4																															1F66655A3C/F-V-031
4																					D										1F663A6859/F-V-032
																															1F64163037/F-V-033
																															1F72107B64/F-V-034
																															1F63467B3D/F-V-035
																															1F645B1111/F-V-036
																															1F61524668/F-V-037
																															1F6132321C/F-V-038
																															1F655A0E14/F-V-039

624 ✱ marked on card as found dead 17 Feb 95 cw

or Animal Caretakers: Contact LTC Anderson, Office Ext 4933, Fax Ext 2152, Home (301) 473-5059 if you have any questions

Discard dead animals

Use scanner to check chip number of dead mice

Mark number of animals alive in each cage

126

see page 123

REDACTED

Project: Active Immunization F1-V Alhydorgel

Notebook #: 3598

Inoculum: Yersinia pestis strain CO92

Route: aerosol

Dose: RUN 1 = 80 LD50 RUN 2 = 104 LD50

REDACTED

Swiss Webster Age: REDACTED at 7-8wk Vendor: Harlan Sprague Dawley Sex: female

Day -	24	25	26	27	28	1						7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Day postinfection	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29

Group	Cage#		Comments/Chip #
Alh alone	1	RUN 1	1F6134004C/F-V-040
GP5	2		1F656C5937/F-V-041
	3		1F61135D10/F-V-042
	4		1F6655574F/F-V-043
	5		1F664B210F/F-V-044
	6	RUN 2	7F7B0A2C5B/F-V-045
	7		1F63737516/F-V-046
	8		1F66240C4B/F-V-047
	9		1F5A6F0C0C/F-V-048
	10		1F65354106/F-V-049
Alh+F1	11		1F76011A50/F-V-050
10 µg	12		1F653D7946/F-V-051
GP6	13		1F624D6A48/F-V-052
	14	RUN 1	1F64030B6F/F-V-053
	15		1F6E295D6D/F-V-054
	16		1F64303B12/F-V-055
	17		1F635D326F/F-V-056
	18		1F6726084C/F-V-057
	19		1F6317796E/F-V-058
BLUE	20		1F64024437/F-V-059
Alh+F1	21	Died during bleeding of 17FEB95	~~1F66056C0A/F-V-060~~
10 µg	22		1F637E6917/F-V-061
urea	23		1F64653F59/F-V-062
GP7	24	RUN 1	1F643F7846/F-V-063
	25		1F6412204B/F-V-064
	26		7F7D261230/F-V-065
	27		1F6132735B/F-V-066
	28		1F635C5D45/F-V-067
	29		1F636A1103/F-V-068
GREEN	30		1F5F542F7F/F-V-069
Alh+F1-V	31		1F6729527F/F-V-070
18.5 µg	32		1F63280353/F-V-071
GP8	33		1F600B6D09/F-V-072
	34	RUN 2	1F77037275/F-V-073
	35		1F615E071B/F-V-074
	36		1F646F0D01/F-V-075
	37		1F66415A60/F-V-076
	38		1F65566046/F-V-077
	39		1F64495262/F-V-078
RED	40		1F6467682E/F-V-079
Alh+F1-V	41		200905054D/F-V-080
37 µg	42		1F636F2F60/F-V-081
GP9	43		1F650E541A/F-V-082
	44	RUN 2	1F637B3E45/F-V-083
	45		1F673C5668/F-V-084
	46		1F647E2E51/F-V-085
	47		1F663B0937/F-V-086
	48		1F62111658/F-V-087
	49		1F6320124C/F-V-088
BLACK	50		1F635A1F05/F-V-089

For Animal Caretakers: Contact LTC Anderson, Office Ext 4933, Fax Ext 2152, Home (301) 473-5059 if you have any questions

Discard dead animals

Use scanner to check chip # of dead animals

Mark number of mice alive

Attn: LTC Anderson

see page 123

Exhibit GA3

REDACTED

Project: Active Immunization F1-V Alhydrogel/CFA

Book #: 3598

Yersinia pestis strain C12

Dose:

Swiss Webster

Age: Arrive

Vendor: Harlan Sprague Dawley

Sex: female

Day -	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	1
Postinfection	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29

Cage#	Comments/Chip #
51	1F683C0B32/F1-VB-061
52	1F615F021F/F1-VB-062
53	1F71772257/F1-VB-063
54	1F644F713D/F1-VB-064
55	1F67412415/F1-VB-065
56	1F66606C2F/F1-VB-066
57	1F77087969/F1-VB-067
58	1F637D1F62/F1-VB-068
59	1F61172742/F1-VB-069
60	1F64511E0E/F1-VB-070
61	1F5756496B/F1-VB-071
62	1F681C4617/F1-VB-072
63	1F68571012/F1-VB-073
64	1F682F6763/F1-VB-074
65	1F673E4577/F1-VB-075
66	1F62681205/F1-VB-076
67	1F655F1E7F/F1-VB-077
68	1F65523377/F1-VB-078
69	1F61284810/F1-VB-079
70	1F6152307E/F1-VB-080
71	1F68382A17/F1-VB-081
72	1F686B1876/F1-VB-082
73	1F60106E03/F1-VB-083
74	1F63242D2D/F1-VB-084
75	1F637A7311/F1-VB-085
76	1F631F0D52/F1-VB-086
77	1F6378473F/F1-VB-087
78	1F63390C39/F1-VB-088
79	1F66517931/F1-VB-089
80	1F642C272A/F1-VB-090
81	1F654E703E/F1-VB-091
82	1F64134129/F1-VB-092
83	1F63355772/F1-VB-093
84	1F621A0263/F1-VB-094
85	1F64361A2D/F1-VB-095
86	1F61774A3F/F1-VB-096
87	1F65276075/F1-VB-097
88	1F646F0905/F1-VB-098
89	1F624C181B/F1-VB-099
90	1F684E3C6F/F1-VB-100
91	1F64034B2F/F1-VB-101
92	1F65083C38/F1-VB-102
93	1F65792E55/F1-VB-103
94	1F6012412E/F1-VB-104
95	1F68321F28/F1-VB-105
96	1F6E313909/F1-VB-106
97	1F614D6F44/F1-VB-107
98	1F62394402/F1-VB-108
99	1F627B5C28/F1-VB-109
100	1F64165A0D/F1-VB-110
101	1F630B4231/F1-VB-111
102	1F6629450D/F1-VB-112
103	1F5F630D12/F1-VB-113
104	1F657C5B25/F1-VB-114
105	1F6337596E/F1-VB-115
106	1F66297260/F1-VB-116
107	1F66524F5A/F1-VB-117
108	1F6829537D/F1-VB-118
109	1F64616E2E/F1-VB-119
110	1F61517936/F1-VB-120

Died During Aerosol

Died During Aerosol

Animal Caretakers: Contact LTC Anderson, Office Ext 4933, Fax Ext 2152, Home (301) 473-5059 if you have any questions

Remove dead animals

Scanner to check chip # of dead animals

Number of mice alive

Remove out of holder

see page 123

Date: [redacted] Project: Active Immunization F1-V Alhydrogel/CFA

Notebook #: 3598

Inoculum: Yersinia pestis strain C12

Route: aerosol Dose: RUN2 = 99 LD50 RUN3 = 129 LD50

[redacted] iss Webster Age: [redacted] at 7-8wk Vendor: Harlan Sprague Dawley Sex: female

Group	Cage#	Day postinfection 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	Comments/Chip #
CFA alone	111	1	1	1	D																											1F65136504/F1-VB
GP23	112	1	1	1	1	D																										1F66002A51/F1-VB
	113	1	1	1	D																											1F5F7D1075/F1-VE
	114	1	1	1	1	D																										1F65074134/F1-VB
	115	1	1	1	1	D																										1F71724836/F1-VB
	116	1	1	1	1	D																										1F656C4F41/F1-VE
	117	1	1	1	1	D																										1F60182148/F1-VB
	118	1	1	1	D																											1F6866573C/F1-VB
	119	1	1	1	1	D																										1F62780205/F1-VB
	120	1	1	1	D																											1F65080173/F1-VB

MTD = 50 RUN2 RUN3

For Animal Caretakers: Contact LTC Anderson, Office Ext 4933, Fax Ext 2152, Home (301) 473-5059 if you have any questions
Discard dead animals
Use scanner to check chip # of dead animals
Mark number of mice alive

See page 127

Date: REDACTED | Project: Active Immunization F1-V Alhydrogel/CFA

Notebook #: 3598

Inoculum: Yersinia pestis strain C12

Route: SC | Dose:

REDACTED viss Webster | Age: REDACTED at 7-8wk | Vendor: Harlan Sprague Dawley | Sex: female

Day postinfection		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	Comments/Chip #
Group	Cage#																															
Alh alone	GP10									D																						1F646A0D06/F-V-090
									D																							7F7B107C58/F-V-091 or 1F6:
																												D				1F6E345D62/F-V-092 OR 1F
										D																						1F64141752/F-V-093 OR 1F6
																D																1F65020D6D/F-V-094 OR 9F
																D																1F62032B51/F-V-095 OR 1F6
									D																							1F635D4B56/F-V-096 OR 7F
										D																						7F7B06623C/F-V-097 OR 7F
										D																						7F7D23252D/F-V-098
											D																					1F630A7004/F-V-099
Alh+F1-V 27 µg	GP11						D																									1F6458061F/F1-VB-001
									D																							1F66294012/F1-VB-002
									D																							1F65323119/F1-VB-003
																																1F68597E22/F1-VB-004
								D																								1F663B023E/F1-VB-005
									D																							1F64742F5A/F1-VB-006
																																1F62713757/F1-VB-007
																																1F664A1B16/F1-VB-008
																											D					1F624C3D76/F1-VB-009
							D																									1F60466754/F1-VB-010
CFA+V 10 µg	GP12																															1F650D343B/F1-VB-011
																																1F640F0668/F1-VB-012
																																1F684A6D42/F1-VB-013
																																1F627B4440/F1-VB-014
																																1F66362421/F1-VB-015
																																1F64121C4F/F1-VB-016
																																1F647A4340/F1-VB-017
																																1F757C7977/F1-VB-018
																																1F65625644/F1-VB-019
																																1F65662E68/F1-VB-020
CFA+V 10 µg urea	GP13	Found dead, 13JAN95, cause unknown																														1F655A455D/F1-VB-021
																																1F63624B51/F1-VB-022
																																1F65487440/F1-VB-023
																																1F61185F09/F1-VB-024
																																1F63296273/F1-VB-025
																																1F6626094C/F1-VB-026
																																1F622A1C39/F1-VB-027
																																1F6122312D/F1-VB-028
																																1F6329775E/F1-VB-029
																																1F64652276/F1-VB-030
CFA-F1-V 27 µg	GP14									D																						1F73061751/F1-VB-031
																		D														1F63672C6B/F1-VB-032
										D																						1F68406158/F1-VB-033
											D																					1F6359061F/F1-VB-034
										D																						1F653E2C12/F1-VB-035
													D																			1F60524B64/F1-VB-036
										D																						1F67274A09/F1-VB-037
							D																									200F13231B/F1-VB-038
																																1F620B7B79/F1-VB-039
																																1F66121653/F1-VB-040
CFA-F1-V 54 µg	GP15										D																					1F66247661/F1-VB-041
											D																					1F652F0D40/F1-VB-042
												D																				1F63103B33/F1-VB-043
												D																				1F673D0C31/F1-VB-044
																																1F62757218/F1-VB-045
												D																				1F63401727/F1-VB-046
													D																			1F665F5D3F/F1-VB-047
																																1F66585F44/F1-VB-048
																																1F61195611/F1-VB-049
																																200737366C/F1-VB-050

For Animal Caretakers: Contact LTC Anderson, Office Ext 4933, Fax Ext 2152, Home (301) 473-5059 if you have any questions

Discard dead animals

Use scanner to check chip # of dead animals

Mark number of mice alive

130

see page 123

REDACTED

Project: Active Immunization F1-V Alhydrogel/CFA

Notebook #: 3598

Inoculum: Yersinia pestis strain C12

Route: SC

Dose:

REDACTED

Swiss Webster

Age: REDACTED at 7-8wk

Vendor: Harlan Sprague Dawley

Sex: female

REDACTED

Day -	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	1
Day postinfection	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29

Group	Cage#	Comments/Chip #
CFA alone	GP16	1F614E2012/F1-VB-051
		1F64297C58/F1-VB-052
		1F75463175/F1-VB-053
		1F626E157C/F1-VB-054
		1F650F5419/F1-VB-055
		1F64075C1A/F1-VB-056
		1F756A3151/F1-VB-057
		1F6121124D/F1-VB-058
		1F655E405E/F1-VB-059
		1F65233C1D/F1-VB-060

DEAD 1 REDACTED

For Animal Caretakers: Contact LTC Anderson, Office Ext 4933, Fax Ext 2152, Home (301) 473-5059 if you have any questions

Discard dead animals

Use scanner to check chip # of dead animals

Mark number of mice alive

Exhibit GA4

REDACTED

ELISA, F1 & V ANT.

STARTING DILUTION 1:640, SERIAL 1:2 DILUTIONS

F1 AND V ANTIGENS (

F1 PLATE #	SERUM #	V PLATE #	F1 PLATE #	SERUM #	V PLATE #
1A F	1117	[redacted] 16A	11A	[redacted] 1844	26A
B	1118	[redacted] 16B	B	[redacted] 1845	B
2A	1119	[redacted] 17A	12A	[redacted] 1846	27A
B	1230	[redacted] 17B	B	[redacted] 1847	B
3A	1231	[redacted] 18A	13A	[redacted] 1848	28A
B	1232	[redacted] 18B	B	[redacted] 1849	B
4A	1233	19A	14A	[redacted] 1850	29A
B	1234	19B	B	[redacted] NEG (N.M.)	B
5A	1235	20A	[redacted] 15A	F1+	30A
B	~~1236~~ 1836 (F1)	20B	B	831	B
6A	~~1237~~ 1837 (F1)	21A			
B	~~1238~~ 1838 (F1)	21B			
7A	[redacted] ~~1836~~ 1236 (F1)	22A			
B	~~1837~~ 1237 (F1)	22B			
8A	~~1838~~ 1238 (F1)	23A			
B	[redacted] 1839	23B			
9A	[redacted] 1840	24A			
B	[redacted] 1841	24B			
10A	[redacted] 1842	25A			
B	[redacted] 1843	25B			

PLATE 1-3 (F1):

200µl 10 10

100 110 110

REMOVED BUFFER FROM COLUMNS 2-12

ADDED 100µl EACH SAMPLE TO COLUMN 1, 100µl BUFFER TO COLUMNS 2-12, & RETITRATED SAMPLES.

1836	POOL GP10	Alhydrogel alone
1837	POOL GP11	Alh+27ugF1-V
1838	POOL GP12	CFA+10ugV
1839	POOL GP13	CFA+10ugV,urea
1840	POOL GP14	CFA+27ugF1-V
1841	POOL GP15	CFA+54ugF1-V
1842	POOL GP16	CFA alone
1843	POOL GP17	Alhydrogel alone
1844	POOL GP18	Alh+27ugF1-V
1845	POOL GP19	CFA+10ugV
1846	POOL GP20	CFA+10ugV,urea
1847	POOL GP21	CFA+27ugF1-V
1848	POOL GP22	CFA+54ugF1-V

REDACTED

SUMMARY

				F1	V
Day63	active F1-V			0	0
Day63	active F1-V			40960	81920
Day63	active F1-V			0	1310720
Day63	active F1-V			0	655360
Day63	active F1-V			163840	1310720
Day63	active F1-V			163840	1310720
Day63	active F1-V			0	1280 R
Day63	active F1-V			0	2560 R
Day63	active F1-V			40960	81920
Day63	active F1-V			0	655360
Day63	active F1-V			0	1310720
Day63	active F1-V			163840	655360
Day63	active F1-V			327680	327680

Data on page 131 is the first direct evidence that the F1-V fusion protein can induce an immune response to both the F1 and V portion of the F1-V fusion protein. This is the first proof of the concept of making a fusion protein which could be used as an immunogen in a future plague vaccine. There was some non-specific reactivity toward V, but low levels compared to the F1-V treated animals.
The second part of the proof will be the protection studies started on REDACT and REDACTED If the protection studies work and the C092 challenge already shows protection, then the fusion protein which is unique could significantly reduce the complexities of the manufacturing process. F1-V forming an aggregate, may also increase the immunogenicity of V.

REDACTED
David M. Heath
Gerald P. Andrews

Summary REDACTED

Serum#	Group	Treatment	Bleed Date	Day Post	F1 Titer	V Titer		Change(wells)
1836	POOL GP10	Alhydrogel alone	REDACTED	Day63	0	0	0	0
1837	POOL GP11	Alh+27ugF1-V		Day63	40960	81920	163840	1
1838	POOL GP12	CFA+10ugV		Day63	0	1310720	1310720	0
1839	POOL GP13	CFA+10ugV,urea		Day63	0	655360	1310720	1
1840	POOL GP14	CFA+27ugF1-V		Day63	163840	1310720	1310720	0
1841	POOL GP15	CFA+54ugF1-V		Day63	163840	1310720	1310720	0
1842	POOL GP16	CFA alone		Day63	0	1280	1280	0
1843	POOL GP17	Alhydrogel alone		Day63	0	2560	0	-2
1844	POOL GP18	Alh+27ugF1-V		Day63	40960	81920	163840	1
1845	POOL GP19	CFA+10ugV		Day63	0	655360	1310720	1
1846	POOL GP20	CFA+10ugV,urea		Day63	0	1310720	1310720	0
1847	POOL GP21	CFA+27ugF1-V		Day63	163840	655360	1310720	1
1848	POOL GP22	CFA+54ugF1-V		Day63	327680	327680	1310720	2
1849	POOL GP23	CFA alone		Day63	0	10240	1280	-3
1850	POOL GP24	CFA+27ugF1-V		Day63	163840	163840	1310720	3
				Controls:				
				F1+ Pool	40960	20480	10240	-1
				831	0	163840	655360	2
				Normal Mouse	0	5120	0	-4

see page 131 & 123

file - Pitt-aerosol data. REDACTED

EXPT. #3: REDACT Aerosol and sc Plague Challenge Expt. (mice)
FOR ACTIVE F1-V IMMUNIZATION CHALLENGE 3 MAR 95 –
AEROSOL - SEE PAGES 127 - 130

suspension	Target Conc./ml	no. CFU/ml	Calculated inhaled no. CFU	No. LD50s
Prespray	1.75x10e10/ml	2.8 x 10e10/ml		
C092/C12				
AGI				
# 1		2.5 x 10e8/ml		
# 2		2.6 x 10e8/ml		
# 3		3.4 x 10e8/ml		

see page 135 for LD50 figures

SUBCUTANEOUS -
Target

Date: REDACTED

PI: LTC Anderson
Agent: Plague
Strain: C12

Animal Model: Mouse

C12 LD50=1.1E+05

Wt: (Ave.) : 27.69

see pgs 127-130

Sex: female

AGI/ml	AGI	cfu/l aerosol	MV	Inhaled Dose cfu	LD50s	Strain
2.50E+08	2.50E+09	4.17E+07	0.025	1.04E+07	94.70	C12
2.60E+08	2.60E+09	4.33E+07	0.025	1.08E+07	98.48	C12
3.40E+08	3.40E+09	5.67E+07	0.025	1.42E+07	128.79	C12

Mouse weight
27.0
32.2
23.4
28.1
25.6
25.6
29.0
30.5
30.1
35.4
27.69 g average

Summary REDACTED

File: Serumbook Treatment | Bleed date | File Update: REDACTED Bleed day | Protocol | F1 TITER | V TITER

#	Group	Treatment	Bleed date	Bleed day	Protocol	F1 TITER	V TITER
220	GP24	F1-V, day 14 antibody	REDACTED	DAY14	F1-V ANTIBODY ONTOGENY	81920	40960
221	GP24	F1-V, day 14 antibody		DAY14	F1-V ANTIBODY ONTOGENY	20480	40960
222	GP24	F1-V, day 14 antibody		DAY14	F1-V ANTIBODY ONTOGENY	81920	20480
223	GP24	F1-V, day 14 antibody		DAY14	F1-V ANTIBODY ONTOGENY	20480	10240
224	GP24	F1-V, day 14 antibody		DAY14	F1-V ANTIBODY ONTOGENY	40960	640
225	GP24	F1-V, day 14 antibody		DAY14	F1-V ANTIBODY ONTOGENY	10240	0
226	GP24	F1-V, day 14 antibody		DAY14	F1-V ANTIBODY ONTOGENY	40960	10240
227	GP24	F1-V, day 14 antibody		DAY14	F1-V ANTIBODY ONTOGENY	40960	40960
228	GP24	F1-V, day 14 antibody		DAY14	F1-V ANTIBODY ONTOGENY	20480	10240
229	GP24	F1-V, day 14 antibody		DAY14	F1-V ANTIBODY ONTOGENY	20480	20480
					Geomean(of positive values only!)	31042	13934
340	GP24	F1-V, day 14 antibody	REDACTED	DAY28	F1-V ANTIBODY ONTOGENY	163840	327680
341	GP24	F1-V, day 14 antibody		DAY28	F1-V ANTIBODY ONTOGENY	81920	327680
342	GP24	F1-V, day 14 antibody		DAY28	F1-V ANTIBODY ONTOGENY	81920	1280
343	GP24	F1-V, day 14 antibody		DAY28	F1-V ANTIBODY ONTOGENY	81920	1310720
344	GP24	F1-V, day 14 antibody		DAY28	F1-V ANTIBODY ONTOGENY	20480	2560
345	GP24	F1-V, day 14 antibody		DAY28	F1-V ANTIBODY ONTOGENY	81920	327680
346	GP24	F1-V, day 14 antibody		DAY28	F1-V ANTIBODY ONTOGENY	327680	655360
347	GP24	F1-V, day 14 antibody		DAY28	F1-V ANTIBODY ONTOGENY	81920	40960
348	GP24	F1-V, day 14 antibody		DAY28	F1-V ANTIBODY ONTOGENY	40960	81920
349	GP24	F1-V, day 14 antibody		DAY28	F1-V ANTIBODY ONTOGENY	81920	163840
					Geomean(of positive values only!)	81920	94101
768	GP24	CFA+27ugF1-V	REDACTED	Day63	active F1-V	327680	1310720
769	GP24	CFA+27ugF1-V		Day63	active F1-V	655360	655360
770	GP24	CFA+27ugF1-V		Day63	active F1-V	327680	655360
771	GP24	CFA+27ugF1-V		Day63	active F1-V	327680	1310720
772	GP24	CFA+27ugF1-V		Day63	active F1-V	655360	655360
773	GP24	CFA+27ugF1-V		Day63	active F1-V	327680	1310720
774	GP24	CFA+27ugF1-V		Day63	active F1-V	163840	40960
775	GP24	CFA+27ugF1-V		Day63	active F1-V	163840	1310720
776	GP24	CFA+27ugF1-V		Day63	active F1-V	655360	1310720
777	GP24	CFA+27ugF1-V		Day63	active F1-V Died		
					Geomean(of positive values only!)	353914	707828

Summary REDACTED

Serum #	Group	File: Serumbook Treatment	Bleed date	File Update: REDACTED Bleed day	Protocol	CHIP #	MISC	V TITER
1688	GP16	CFA alone	REDACTED	Day63	active F1-V	1F614E2012/F1-VB-051		O
1689	GP16	CFA alone		Day63	active F1-V	1F64297C58/F1-VB-052		O
1690	GP16	CFA alone		Day63	active F1-V	1F75463175/F1-VB-053		O
1691	GP16	CFA alone		Day63	active F1-V	1F626E157C/F1-VB-054		O
1692	GP16	CFA alone		Day63	active F1-V	1F650F5419/F1-VB-055		O
1693	GP16	CFA alone		Day63	active F1-V	1F64075C1A/F1-VB-056		O
1694	GP16	CFA alone		Day63	active F1-V	1F756A3151/F1-VB-057		No sample
1695	GP16	CFA alone		Day63	active F1-V	1F6121124D/F1-VB-058		640
1696	GP16	CFA alone		Day63	active F1-V	1F655E405E/F1-VB-059		O
1697	GP16	CFA alone		Day63	active F1-V	1F65233C1D/F1-VB-060		O
							Geomean(of positive values only!)	640
1758	GP23	CFA alone	REDACTED	Day63	active F1-V	1F65136504/F1-VB-121		1280
1759	GP23	CFA alone		Day63	active F1-V	1F66002A51/F1-VB-122		O
1760	GP23	CFA alone		Day63	active F1-V	1F5F7D1075/F1-VB-123		20480
1761	GP23	CFA alone		Day63	active F1-V	1F65074134/F1-VB-124		O
1762	GP23	CFA alone		Day63	active F1-V	1F71724836/F1-VB-125		640
1763	GP23	CFA alone		Day63	active F1-V	1F656C4F41/F1-VB-126		O
1764	GP23	CFA alone		Day63	active F1-V	1F60182148/F1-VB-127		O
1765	GP23	CFA alone		Day63	active F1-V	1F6866573C/F1-VB-128		O
1766	GP23	CFA alone		Day63	active F1-V	1F62780205/F1-VB-129		1280
1767	GP23	CFA alone		Day63	active F1-V	1F65080173/F1-VB-130		O
							Geomean(of positive values only!)	2153

Controls:	
Normal Mouse	0
F1+ Pool	5120
Serum 831	327680

CFA appears to increase background problems. #1760 is so high it appears to be an error of some sort. Most probable, a tube contaminant or mislabeled tube.

Summary REDACTED

Serum #	Group	File: Serumbook Treatment	Bleed date	File Update: REDACTED Bleed day	Protocol	F1 TITER	V TITER
2081	GP1 Pool	Alhydrogel only	REDACTED	Day63	ALH-F1-V fusion	O	O
2082	GP2 Pool	ALH+10µgF1		Day63	ALH-F1-V fusion	81920	O
2083	GP3 Pool	ALH+10µgF2urea		Day63	ALH-F1-V fusion	81920	O
2084	GP4 Pool	ALH+18.5µg F1-V		Day63	ALH-F1-V fusion	81920	163840
2085	GP5 Pool	Alhydrogel only		Day63	ALH-F1-V fusion	O	O
2086	GP6 Pool	ALH+10µgF1		Day63	ALH-F1-V fusion	40960	O
2087	GP7 Pool	ALH+10µgF2urea		Day63	ALH-F1-V fusion	81920	O
2088	GP8 Pool	ALH+18.5µg F1-V		Day63	ALH-F1-V fusion	81920	327680
2089	GP9 Pool	ALH+37µg F1-V		Day63	ALH-F1-V fusion	163840	163840

AEROSOL EXPOSURE SHEET

Aerosol exposure #: 95-029H

Date: REDACTED

Aerosol Operator: SGT David A. McCaskey

Pre-operational Check Performed: OK Dry T: 81 Wet T: 74 Rel. Hum.: 72%

Aerosol System: Nose Only

System Flow Rate: 12 L/min

Collison #: B

Agent: Plague

Protocol #:

P.I.: Anderson

Run #	Animal #	Animal Species	Start T Dry	Start T Wet	Start T Rel. Hum.	5 min T Dry	5 min T Wet	5 min T Rel. Hum.	Start Time	AGI #	Comments
1	20	Mice	80	62	35%	80	72	69%	1025	10	C12 50 LD50
2	25	Mice	80	62	35%	80	74	76%	1036	30	C12 50 LD50
3	25	Mice	80	64	42%	80	74	76%	1127	20A	C12 50 LD50

Jennifer M. Conroy

see page 127-128

AEROSOL EXPOSURE SHEET

Aerosol exposure #: 95-032H

Date: REDACTED

Aerosol Operator: RT

Pre-operational Check Performed: ✓ Dry T: 82 Wet T: 72 Rel. Hum.: 64%

Aerosol System: NOSE-ONLY

System Flow Rate: 12LPM

Collison #: B

Agent: PLAGUE

Protocol #:

P.I.: LTC ANDERSON

Run #	Animal #	Animal Species	Start T Dry	Start T Wet	Start T Rel. Hum.	5 min T Dry	5 min T Wet	5 min T Rel. Hum.	Start Time	AGI #	Comments
1	~~20~~ 19	MICE	82	70	56%	82	74	70%	1035	50	C12 50 LD50
2	20	"	82	68	50%	82	74	70%	1101	3	C12 MAX Imse
											escaped / 1 min loss exp. marked in black on
3	—	—			HEPA		WASH				
4	20	MICE	82	68	50%	82	75	72%	~~1140~~ 1147	37A	C092 50 LD50
5	20	mice	82	69	52%	82	74	72%	1204	7	C092 MAX

K. Jammeville

REDACTED

Exhibit GA5

Dear Dr Friedlander

CBDE/USAMRIID COLLABORATIVE RESEARCH INTO PROTECTIVE EFFICACY OF RECOMBINANT V-ANTIGEN AGAINST PARENTERAL AND AEROSOL CHALLENGE WITH YERSINIA PESTIS

As you are aware, CBDE has data to suggest that the V-antigen of the plague causing organism *Yersinia pestis*, when used as an immunogen, is highly protective against plague. The V-antigen could therefore be a major component of an improved plague vaccine to be developed in the future by CBDE.

You recently indicated to us that USAMRIID wished to collaborate in testing the protective capacity of the V-antigen against parenteral and aerosol challenge with virulent plague. We agreed that such a collaboration would be desirable because it could generate valuable data which would be of benefit to both CBDE and USAMRIID. We therefore decided that the collaboration should, in the future, be the subject of a Project Arrangement under the Memorandum of Understanding between the Secretary of Defense (US) and the Secretary of State for Defence (UK) concerning Technology Research and Development Projects (which is currently still under negotiation).

However, we also agreed that any delay in the collaboration would reduce the benefit of the resulting data, and therefore it would be desirable to commence work in advance of a more formal Project Arrangement.

Accordingly, this letter sets out below the respective duties, rights and responsibilities of each of us under the collaboration, *pro tem*, pending the negotiation of a more comprehensive arrangement:

1. SCOPE OF WORK

a. CBDE will supply to USAMRIID, for the purposes described in (b), the following:

i. 30 mg of recombinant *Yersinia pestis* V-antigen.

ii. Protocols detailing the immunisation route, doses and schedules used at CBDE.

iii. Polyclonal antisera raised against the V antigen of *Yersinia pestis*.

iv. Details of the CBDE challenge route, challenge strain and protection data afforded by the V-antigen vaccine against parenteral challenge with *Yersinia pestis*.

b. USAMRIID will:

i. Immunise groups of animals parenterally with the following:

- V-antigen in combination with Alhydrogel.

Exhibit GA6

Suggested protocol for the F1-whole V fusion protein.

File: F1-wholeV fusion last update REDACTED
Protocol: B95-01
F1-WV fusion protein immunization and challenge

Investigators: CPT Heath, Dr. Welkos, LTC Anderson, COL Friedlander

Background. CPT David Heath produced and purified a recombinant F1-V fusion protein. The protein is positive by Western blot to F1 and V. Only part of the V-protein was used in the intial F1-V fusion. This F1-V fusion was immunogenic, but was not very efficacious when compared to the whole V-protein (WV) produced by Mauro to protect against F1 minus strains of *Y. pestis*. This is a repeat of part of the intital F1-V protection study using the whole V-protein in the F1-WV fusion. Subcutaneous injection along back of the mouse for immunization.

Purpose: Immunize and challenge mice to check on immunogenicity and protection against the CO92 and C12 strain of Y. pestis by sc and aerosol challenge, **100-Max LD_{50}**.

Alhydrogel, 1.3%, from SuperFos. Batch # 2043, Expiration date None, _____µg of AL/dose

Endotoxin level in the F1-WV preparation is _____ U/ml.

Will use **Mauro's V** which has been urea treated as per the F1-WV procedure. Details in CPT Heath's laboratory notebook.

Immunization Groups: 10 Swiss Webster female mice per group from Harlan Sprague Dawley

Implantable Micro Identification transponders from: BioMeic Data Systems, Inc 255 W. Spring Valley Ave. Maywod, NJ 07607, 1-800-526-BMDS

		Strain	Dose LD_{50}	# Mice
Subcutaneous challenge				
Group 1	Alhydrogel alone, days 0, 30, sc	C12	100	10
Group 2	Alhydrogel + 13.6 µg F1-WV fusion protein day 0, 30, sc	C12	100	10
Group 3	Alhydrogel + 10 µg Mauro-V urea, days 0, 30,sc	C12	Max	10
Group 4	Alhydrogel + 13.6 µg F-1WV fusion protein days 0,30,sc	C12	Max	10
Group 5	Alhydrogel + 27.2 µg F1-WV fusion protein days 0, 30, sc	C12	Max	10
Group 6	Alhydrogel alone days 0, 30, sc	C12	Max	10
Group 7	Alhydrogel + 13.6 µg F1-WV fusion protein day 0, 30, sc	CO92	100	10
Group 8	Alhydrogel alone, days 0, 30, sc	CO92	100	10
Aerosol challenge				
Group 09	Alhydrogel alone, days 0, 30, sc	C12	50	10
Group 10	Alhydrogel + 13.6 µg F1-WV fusion protein day 0, 30, sc	C12	50	10
Group 11	Alhydrogel + 10 µg Mauro-V urea, days 0, 30, sc	C12	Max	10
Group 12	Alhydrogel + 13.6 µg F1-WV fusion protein, days 0, 30, sc	C12	Max	10
Group 13	Alhydrogel + 27.2 µg F1-WV fusion protein days 0,30, sc	C12	Max	10
Group 14	Alhydrogel alone, days 0, 30, sc	C12	Max	10
Group 15	Alhydrogel + 13.6 µg F1-WV fusion protein days 0, 30, sc	CO92	100	10
Group 16	Alhydrogel alone days 0, 30, sc	CO92	100	10
~~Group 17~~	~~Alhydrogel + 13.6 µg F1-WV fast prep, days 0, 30, sc~~	~~C12~~	~~Max~~	~~10~~
Group 18	Alhydrogel + 10 µg F1 + 10 µg Mauro's V, days 0, 30, sc	C12	Max	10
Group 19	Greer plague vaccine, days 0, 30, sc	C12	Max	~~10~~ 05
Group 20	Alhydrogel alone, day 0, 30, sc	C12	Max	05

180

10	Group 21	ALH + 13.6 µg F1-WV fusion protein day 0, 30, sc --antibody response Measure titer at 7,14, 27,57, 90		10
	~~Group 22~~	~~ALH + 13.6 µg F1-WV fusion protein day 0, 30, sc --lung lavage, day 57~~	x	05
10	Group 23	ALH + 27.2 µg F1-WV fusion protein day 0, 30, sc --antibody response Measure titer at 7, 14, 27, 57, 90		10
	~~Group 24~~	~~ALH + 27.2 µg F1-WV fusion protein day 0, 30, sc --lung lavage, day 57~~	x	05
10	~~Group 25~~	~~ALH + Mauro-V urea, 10 ug, day 0, 30, sc --antibody response~~		10
	~~Group 26~~	ALH + Mauro-V urea, 10 ug, day 0, 30, sc --lung lavage, day 57	x	~~05~~
	~~Group 27~~	~~ALH alone, day 0,30, sc~~ ~~Measure titer at 7, 14, 27,57, 90~~		10
	~~Group 28~~	~~ALH alone, day 0,30, sc, --lung lavage, day 57~~	x	05
10	Group29	ALH alone, for spleen weights 28 day pi		~~10~~ 05
			Total	220

Schedule

Groups 1-20

13Jun95	Arrival of Swiss Webster mice, female 7-8 wks, Harlan Sprague Dawley in AA-3 (Barrier)
24Jun95	Chipped with BioMedic Data Systems transponders, West
27Jun95	1st immunization, day 0
27Jul95	2nd Immunization, day 30
24Aug95	Bleed to determine prechallenge titers, day 58
31Aug95	Challenge by aerosol & sc routes, day 65
28Sep95	Terminal bleed, day 28 pi, titrate spleens# serum #

Group 21-25

13Jun95	Mice arrive
27Jun95	1st immunization, AA-3
11Jul95	Groups 21, 23, 25; Bleed, day 14, AA-3, SERUM#
26Jul95	Groups 21, 23, 25; Bleed, day 29, AA-3, SERUM#
27Jul95	2nd immunization, day
31Aug95	Bleed, day 65, AA-3, Groups SERUM# Groups 22, 24, 26, and 28 for serum titer & bronchial lavage #
28Sep95	Group 29 for Spleen weights #
25Sep95	Groups 21, 23, and 25; day 90, serum #

Chip numbers for all groups extra alhydrogel controls

File: F1-wholeV fusion last update REDACTED
Protocol: B95-01
F1-WV fusion protein immunization and challenge

Investigators: CPT Heath, Dr. Welkos, LTC Anderson, COL Friedlander

Background. CPT David Heath produced and purified a recombinant F1-V fusion protein. The protein is positive by Western blot to F1 and V. Only part of the V-protein was used in the intial F1-V fusion. This F1-V fusion was immunogenic, but was not very efficacious when compared to the whole V-protein (WV) produced by Mauro to protect against F1 minus strains of *Y. pestis*. This is a repeat of part of the intital F1-V protection study using the whole V-protein in the F1-WV fusion. Subcutaneous injection along back of the mouse for immunization.

Purpose: Immunize and challenge mice to check on immunogenicity and protection against the CO92 and C12 strain of Y. pestis by sc and aerosol challenge, **100-Max LD_{50}**.

Alhydrogel, 1.3%, from SuperFos. Batch # 2043, Expiration date None, 0.1857 mg of AL/dose

Endotoxin level in the F1-WV preparation is _____ U/ml.

Will use **Mauro's V** which has been urea treated as per the F1-WV procedure. Details in CPT Heath's laboratory notebook.

Immunization Groups: 10 Swiss Webster female mice per group from Harlan Sprague Dawley

Implantable Micro Identification transponders from: BioMeic Data Systems, Inc 255 W. Spring Valley Ave. Maywod, NJ 07607, 1-800-526-BMDS

Subcutaneous challenge		Strain	Dose LD_{50}	# Mice
Group 1	Alhydrogel alone, days 0, 30, sc	C12	100	10 NC
Group 2	Alhydrogel + 13.6 µg F1-WV fusion protein day 0, 30, sc	C12	100	10
Group 3	Alhydrogel + 10 µg Mauro-V urea, days 0, 30,sc	C12	Max	10
Group 4	Alhydrogel + 13.6 µg F-1WV fusion protein days 0,30,sc	C12	Max	10
Group 5	Alhydrogel + 27.2 µg F1-WV fusion protein days 0, 30, sc	C12	Max	10
Group 6	Alhydrogel alone days 0, 30, sc	C12	Max	10 NC
Group 7	Alhydrogel + 13.6 µg F1-WV fusion protein day 0, 30, sc	CO92	100	10
Group 8	Alhydrogel alone, days 0, 30, sc	CO92	100	10 NC
Aerosol challenge				
Group 09	Alhydrogel alone, days 0, 30, sc	C12	50	10
Group 10	Alhydrogel + 13.6 µg F1-WV fusion protein day 0, 30, sc	C12	50	10
Group 11	Alhydrogel + 10 µg Mauro-V urea, days 0, 30, sc	C12	Max	10
Group 12	Alhydrogel + 13.6 µg F1-WV fusion protein, days 0, 30, sc	C12	Max	10
Group 13	Alhydrogel + 27.2 µg F1-WV fusion protein days 0,30, sc	C12	Max	10
Group 14	Alhydrogel alone, days 0, 30, sc	C12	Max	10
Group 15	Alhydrogel + 13.6 µg F1-WV fusion protein days 0, 30, sc	CO92	100	10
Group 16	Alhydrogel alone days 0, 30, sc	CO92	100	10
Group 17	Alhydrogel + 10 µg F1 + 10 µg Mauro's V, days 0, 30, sc	C12	Max	10
Group 18	Greer plague vaccine, days 0, 30, sc	C12	Max	09 NC
Group 19	Alhydrogel alone, day 0, 30, sc LOT 1013W2	C12	Max	05
Group 20	ALH + 13.6 µg F1-WV fusion protein day 0, 30, sc --antibody response Measure titer at X,14, 27,57, lung lavage day 57			10

Purchased Sandoz in AA-4. Lot 1.8 per cylinder / month. Then in the box when

	Measure titer at ~~X~~, 14, 27, 57; lung lavage day 57	
Group 22	ALH + Mauro-V urea, 10 ug, day 0, 30, sc --antibody response	10
Group 23	ALH alone, day 0,30, sc	10
	Measure titer at ~~X~~ 14, 27,57; lung lavage day 57	
Group 24	Greer plague vaccine days 0, 30, sc	
	Measure titer at ~~X~~, 14, 27, 57; lung lavage day 57 Chipped	05
Group 25	Alhydrogel + 10 µg F1 + 10 µg Mauro's V, days 0, 30, sc	07
	Measure titer at ~~X~~ 14, 27, 57; lung lavage day 57, Mice rec'd 14Jun95	
	Total	232

Schedule

Groups 1-19

13Jun95 Arrival of Swiss Webster mice, female 7-8 wks, Harlan Sprague Dawley in AA-3 (Barrier)

24Jun95 Chipped with BioMedic Data Systems transponders, West

27Jun95 1st immunization, day 0, Zimmerman, West, Giunanzio, Archer, Anderson

25Jul95 2nd Immunization, day 28, Hall, West, Thomason, Archer, Anderson

24Aug95 Bleed to determine prechallenge titers, day 58 5664 - 5847

~~08~~ 06 Sep95 Challenge by aerosol & sc routes, day ~~58~~ 60, AA-3 ~~5548 - 5899~~

~~28Sep~~ 06 OCT 95 Terminal bleed, day 28 pi, titrate spleens# 10020 - 10094 serum # 6052 - 6157

ZIMMERMAN, PLUMMER, ANDERSON, SHAMBLIN

Group 20-23

13Jun95 Mice arrive

27Jun95 1st immunization, AA-3

11Jul95 Groups 20, 21, 22; Bleed, day 14, AA-3, SERUM# 4542 - 4593

26Jul95 Groups 20, 21, 22; Bleed, day 29, AA-3, SERUM# 4807 - 4858 GIANANZIO,

25Jul95 2nd immunization, day28, new barrier, AR-5

~~01~~ 04 Sep95 Bleed, day 65, AA-3, Groups 24, 25 SERUM# 5548 - 5899 GIUNANZIO, HALL

Groups 20, 21, 22, and 23 for serum titer & bronchial lavage #5900 - 5951

~~01~~ 04 Sep95 Group 23 for Spleen weights # 10010 - 10019 ARCHER, DR PULLEN

Chip numbers for all groups extra alhydrogel controls

No Chip GP1
No Chip
No Chip
No Chip
No Chip
No Chip
No Chip
No Chip
No Chip
No Chip
200E2C4363/WV-001 GP2
20103F1F72/WV-002
1F73663C4C/WV-003
1F29341D67/WV-004
2001693A3C/WV-005
2000183C0C/WV-006
1F561F4725/WV-007
1F5F027808/WV-008
1F4B1D3346/WV-009
20023A3371/WV-010

Number of hybridomas in AA-4 has 1.8 per spleen/mouse. This is the last when

1F687D403F/WV-012
1F46376400/WV-013
2041670F29/WV-014
20024E4B45/WV-015
1F6135301B/WV-016
1F734C475B/WV-017
1F575E2C00/WV-018
200E317928/WV-019
1F5718640E/WV-020
1F22052119/WV-021 GP4
200B54374A/WV-022
1F76733B3D/WV-023
201D32642D/WV-024
1F757D303F/WV-025
200D150F2F/WV-026
203C120210/WV-027
200F585326/WV-028
1F667A2859/WV-029
1F56224D1C/WV-030
200B4A612A/WV-031 GP5
201D3B1B6D/WV-032
2041744C5F/WV-033
1F41111877/WV-034
20430C256C/WV-035
1F570E7408/WV-036
1F565A1120/WV-037 Missing chip new 7F7A0E7134E
1F735B6D26/WV-038
1F7F320828/WV-039
2003135E6C/WV-040
No Chip GP6
No Chip
No Chip
No Chip
No Chip
No Chip
No Chip
No Chip
No Chip
No Chip
NoChip
1F73175007/WV-041 GP7
1F63711B72/WV-042
1F7C42653E/WV-043
200B622C47/WV-044
1F7F2E5D57/WV-045
1F66752C5A/WV-046
1F5E776329/WV-047
1F4E420B46/WV-048
200E4D364F/WV-049
203C1D2463/WV-050
No Chip GP8
No Chip
No Chip
No Chip

AA-4 Lev 1.8 exchanges/minute. Take in the top when

No Chip
No Chip
No Chip
No chip
2002537219/WV-051 GP9
201D31583A/WV-052
201D404B38/WV-053
200D22753C/WV-054
1F56211654/WV-055
1F5F154528/WV-056
200A195D60/WV-057
200C043917/WV-058
200142120B/WV-059
1F561F5814/WV-060
201D2D4650/WV-061 GP10
201D30276C/WV-062
1F73085B0B/WV-063
1F757A0A68/WV-064
200F353666/WV-065
203C6C2216/WV-066
200657087B/WV-067
20415E0E33/WV-068
1F501B492D/WV-069
200D7A4D0C/WV-070
1F58195B15/WV-071 GP11
1F7C414D57/WV-072
200A024E06/WV-073
200D34514E/WV-074
1F7F373873/WV-075
2018766969/WV-076
1F61110A65/WV-077
200B0C1732/WV-078
200D51136F/WV-079
1F657E7608/WV-080
20001F4D74/WV-081 GP12
20197C6467/WV-082
1F560B532D/WV-083
2018742331/WV-084
1F28782021/WV-085
203C0C5C3C/WV-086
1F402E787B/WV-087
200B386439/WV-088
200D7E7D58/WV-089
200666175D/WV-090
2019711D39/WV-091 GP12
20017C5211/WV-092
201B493C40/WV-093
1F4E1D383E/WV-094
200B691F4D/WV-095
1F63265C7C/WV-096
1F56147502/WV-097
2041632517/WV-098

weight at time of challenge
34.2
29.0
34.4
41.9
29.8
37.5
28.5
28.3
34.9
37.4
335.9/10 = 33.6 gm avg. weight

Pantoprazole was in AA-4 for 1.8 mi at 4 hours/minute. Then it sat for when

1F760E2120/WV-101 GP14
1F6A15B0B1/WV-102
2037497200/WV-103
200F417110/WV-104
20448E5A84/WV-105
201D462F4F/WV-106
2005033A20/WV-107
1F7D7C4227/WV-108
2035422049/WV-109
201D2E2C69/WV-110
203A555100/WV-111 GP15
1F561E2E3F/WV-112
1F57015930/WV-113
1F5626184D/WV-114
200F31011F/WV-115
201E6C4313/WV-116
2018704C0C/WV-117
1F19690758/WV-118
200F136D51/WV-119
1F76132731/WV-120
201052314D/WV-131 GP16
1F5615492D/WV-132
1F73656128/WV-133
2001311618/WV-134
20104C6E16/WV-135
201C317E15/WV-136
200D48117A/WV-137
200046504A/WV-138
1F62746427/WV-139
201D483546/WV-140
201D2E2A6B/WV-141 GP17
200A05272A/WV-142
2000137756/WV-143
200C06410D/WV-144 – 1F6C7F3343
1F7D60453F/WV-145
2041580443/WV-146
1F75787C78/WV-147
1F20264457/WV-148
1F560D116D/WV-149
1F73671E69/WV-150
No chip GP18
No chip
No chip
No chip
No chip
No chip
No chip
No chip
No chip
2000037B62/WV-122 GP19
1F6679384A/WV-123
20093B4D4F/WV-125

Dimethyl thiobiuret in WA-4: low 1.8 ppm ethylenuren / ammonia. The is the top when

[illegible]/WV-154 GP20
[illegible]/WV-157
[illegible]/WV-158
[illegible]/WV-159
[illegible]/WV-160
[illegible]/WV-161
[illegible]/WV-162
[illegible]/WV-163
[illegible]/WV-164
[illegible]/WV-165
1F[illegible]/WV-166 GP21
1F1F[illegible]D/WV-167
200A112F16/WV-168
1F58244324/WV-169
200D2F170D/WV-170
20097A0857/WV-161
200C032829/WV-162
2010346339/WV-163
1F743C6948/WV-164
201F012818/WV-165
201D480C8F/WV-176 GP22
2035323B3E/WV-177
1F29476B06/WV-178
1F791B202D/WV-179
20415D784A/WV-180
203C137F12/WV-171
1F60390543/WV-172
1F76001259/WV-173
1F56035E2A/WV-174
20447C326E/WV-175
1F7C4C0217/WV-186 GP23
1F203F156D/WV-187
2001361415/WV-188
1F7C2B0733/WV-189
1F73571B7C/WV-190
1F6337794E/WV-181
200B5E4C2B/WV-182
1F60786524/WV-183
20024F226D/WV-184
1F56155B1B/WV-185
200F6B1551/WV-121 GP24 — Pirm assay 95, cause unknown
1F2052056A/WV-124
2009221C19/WV-126
1F2A6A1E2F/WV-128
2008425640/WV-130
No Chip GP25
No Chip
No Chip
No Chip
No Chip
No Chip
No Chip

Pumbers hardline in AA-4 has 1.8 ppm of hexane / mandate. The is the test when

REDACTED

Project: Recombinant F1-whole V Fusion

Notebook #: 3739

Inoculum: Yersinia pestis strain, CO92 ﻃﺎﻋﻮﻥ

Route: sc

Dose:

REDACTED Swiss Webster Arri REDACTED 7-8wks Vendor: Harlan Sprague Dawley Sex: female

REDACTED

Day	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	1	2	3	4	5	6	7	
Day postinfection	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	Comments/Chip #
Group LD50																															
1 C12 Alhydrogel alone						D																									No Chip
							D																								No Chip
							D																								No Chip
								D																							No Chip
								D																							No Chip
									D																						No Chip
									D																						No Chip
									D																						No Chip
											D																				No Chip
												D																			No Chip
2 C12 Alhydrogel 13.6ug F1-WV																															200E2C4363/WV-001
																															20103F1E72/WV-002
																															1F73663C4C/WV-003
																															1F29341D67/WV-004
																															2001693A3C/WV-005
																															2000183C0C/WV-006
																															1F561F4725/WV-007
																															1F5F027808/WV-008
																															1F4B1D3346/WV-009
3 C12 Alhydrogel 10ug Mauro-V																															20023A3371/WV-010
							D																								1F174C5727/WV-011
																															~~1F657D409F/WV-012~~
																															1F46376400/WV-013
																															2041670F29/WV-014
																															20024E4B45/WV-015
																															1F6135301B/WV-016
																															1F734C475B/WV-017
																															1F575E2C00/WV-018
																															200E317928/WV-019
																															1F5718640E/WV-020
4 C12 Alhydrogel 13.6ug F1-WV																															1F22052119/WV-021
																															200B54374A/WV-022
																															1F76733B3D/WV-023
																															201D32642D/WV-024
																															1F757D303F/WV-025
							D																								~~200D150F2E/WV-026~~
																															203C120210/WV-027
																															200F585326/WV-028
																															1F667A2859/WV-029
5 C12 Alhydrogel 27.2ug F1-WV																															1F56224D1C/WV-030
																															200B4A612A/WV-031
																															201D3B1B6D/WV-032
																															2041744C5F/WV-033
																															1F41111877/WV-034
																															20430C256C/WV-035
																															1F570E7408/WV-036
																															7F7A0E7B4E/WV-037 RE
																															1F735B6D26/WV-038
																															1F7F320828/WV-039
																															2003135E6C/WV-040
6 C12 Alhydrogel alone				D																											~~No Chip~~
				D																											~~No Chip~~
				D																											~~No Chip~~
				D																											~~No Chip~~
																															~~No Chip~~
				D																											~~No Chip~~
				D																											~~No Chip~~
				D																											~~No Chip~~
						D																									~~No Chip~~
						D																									~~No Chip~~
						D																									~~NoChip~~

For Animal Caretakers: Contact LTC Anderson, Office Ext 4933, Fax Ext 2152, Home (301) 473-5059 if you have any questions

Discard dead animals

Use scanner to check chip number of dead mice

Mark number of animals alive in each cage

re = mouse has been rechipped

see page 39

REDACTED

Project: Recombinant F1-wholeV Fusion

Notebook #: 3739

Inoculum: Yersinia pestis strain, CO92 see below

Route: sc

Dose:

REDACTED Swiss Webster

REDACTED at 7-8wks

Vendor: Harlan Sprague Dawley

Sex: female

	Day -	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	1	2	3	4	5	6	7	
Day postinfection		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	Comments/Chip #
Group	LD50																															
7	CO92	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|		1F73175007/WV-04
Alhydrogel		|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|		1F63711B72/WV-04
13.6ug		|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|		1F7C42653E/WV-04
F1-WV		|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|		200B622C47/WV-04
	54 LD50	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|		1F7F2E5D57/WV-04
		|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|		1F66752C5A/WV-04
		|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|		1F5E776329/WV-04
		|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|		1F4E420B46/WV-04
		|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|		200E4D364F/WV-04
		|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|		203C1D2463/WV-05
8	Co92	|	|	|	D																											No Chip
Alhydrogel		|	|	|	D																											No Chip
alone		|	|	|	|	D																									No Chip
		|	|	|	|	D																									No Chip
	54 LD50 MTD ± SD 4.8 ± 1.6	|	|	|	|	D																									No Chip
		|	|	|	|	D																									No Chip
		|	|	|	|	|	|	D																							No Chip
		|	|	|	|	|	|	D																							No Chip
		|	|	|	|	|	|	|	D																						No Chip
		|	|	|	|	|	|	|	D																						No chip

For Animal Caretakers: Contact LTC Anderson, Office Ext 4933, Fax Ext 2152, Home (301) 473-5059 if you have any questions

Discard dead animals

Use scanner to check chip number of dead mice

Mark number of animals alive in each cage

re = mouse has been rechipped

[redacted]

Project: Recombinant F1-wholeV Fusion

[redacted]book #: 3739

[redacted]culum: Yersinia pestis strain, CO92 OR C12

[redacted]ute: AEROSOL Dose:

[redacted]wiss Webster A[redacted]at 7-8wks Vendor: Harlan Sprague Dawley Sex: female

[redacted]ay -	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	1	2	3	4	5	6	7	
[redacted]y postinfection	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	Comments/Chip #
[redacted]oup LD50																															
9 C12 [redacted]ydrogel [redacted]ne BLACK WHITE				D																											~~2002537219/WV-051~~
	D	F-D		D		PURPLE CIRCLE																									201D31583A/WV-052 - Died
				D																											~~201D404B98/WV-053~~
				D																											~~200D22753C/WV-054~~
				D																											~~1F56211654/WV-055~~
				D																											~~1F5F154528/WV-056~~
				D																											~~200A195D66/WV-057~~
				D																											~~200C043917/WV-058~~
				D																											~~2001423208/WV-059~~
				D																											~~1F561F5814/WV-060~~
10 C12 [redacted]ydrogel .6ug -WV WHITE WHITE RUN 1																															201D2D4650/WV-061
																															201D30276C/WV-062
																															1F73085B0B/WV-063
																															1F757A0A68/WV-064
																															200F353666/WV-065
																															203C6C2216/WV-066
																															200657087B/WV-067
																															20415E0E33/WV-068
																															1F501B492D/WV-069
																															200D7A4D0C/WV-070
11 C12 [redacted]ydroge Max [redacted]ug [redacted]uro-v [redacted]a WHITE BLACK RUN 2 BLUE RUN 3 RED RUN 4						D																									~~1F58195B15/WV-071~~
								D																							1F7C414D57/WV-072
																															~~200A024E06/WV-073~~
																															200D34514E/WV-074
																															1F7F373873/WV-075
																															2018766969/WV-076
																															1F61110A65/WV-077
																															200B0C1732/WV-078
																															200D51136F/WV-079
																															1F657E7608/WV-080
12 C12 [redacted]ydroge Max .6ug -WV RED BLACK RUN 2 BLUE RUN 3 RED RUN 4																															20001F4D74/WV-081
																															20197C6467/WV-082
																															1F560B532D/WV-083
																															2018742331/WV-084
																															1F28782021/WV-085
																															203C0C5C3C/WV-086
																															1F402E787B/WV-087
																															200B386439/WV-088
																															200D7E7D58/WV-089
																															200666175D/WV-090
13 C12 [redacted]ydroge Max .2ug -WV WHITE BLACK RUN 2 BLUE RUN 3 RED RUN 4																															2019711D39/WV-091
																															20017C5211/WV-092
																															201B493C40/WV-093
																															1F4E1D383E/WV-094
																															200B691F4D/WV-095
																															1F63265C7C/WV-096
																															1F56147502/WV-097
																															2041632517/WV-098
																															1F560E502D/WV-099
																															1F7D1A5E6C/WV-100
14 C12 [redacted]ydrogel Max [redacted]ne GROUP COLOR (SEAL) RUN COLOR (INK) BLACK RUN BLUE RUN 3		F-D		D		PURPLE CIRCLE																									1F720E4120/WV-101 - Died
				D																											~~1F6A163031/WV-102~~
				D																											~~2037497268/WV-103~~
				D																											~~200F44711C/WV-104~~
				D																											~~20446E5A54/WV-105~~
				D																											~~201D452F4F/WV-106~~
				D																											~~2003033A20/WV-107~~
				D																											~~1F7C7C4227/WV-108~~
				D																											~~2035422649/WV-109~~
				±	D																										~~201D2E2C69/WV-110~~

[redacted] Animal Caretakers: Contact LTC Anderson, Office Ext 4933, Fax Ext 2152, Home (301) 473-5059 if you have any questions

[redacted]card dead animals

[redacted]e scanner to check chip number of dead mice

[redacted]rk number of animals alive in each cage

[redacted]= mouse has been rechipped

REDACTED

Project: Recombinant F1-wholeV Fusion

Notebook #: 3739

Inoculum: Yersinia pestis strain, CO92 OR C12

Route: AEROSOL Dose:

REDACTED Swiss Webster A REDACTED t 7-8wks Vendor: Harlan Sprague Dawley Sex: female

Day -	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	1	2	3	4	5	6	7	
Day postinfection	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	Comments/Chip #
Group	LD50																														
15 CO92 Alhydrogel 13.6ug F1-WV																															203A555100/WV-111
																															1F561E2E3F/WV-112
																															1F57015930/WV-113
																															1F5626184D/WV-114
																															200F31011F/WV-115
																															201E6C4313/WV-116
																															2018704C0C/WV-117
																															1F19690758/WV-118
																															200F136D51/WV-119
																															1F76132731/WV-120
16 CO92 Alhydrogel alone				D																											~~201052914D/WV-131~~
				D																											~~1F5615492D/WV-132~~
				D																											~~1F73656128/WV-133~~
				D																											2001311618/WV-134
				D																											~~20104C6E10/WV-135~~
				D																											~~201C317E15/WV-136~~
				D																											~~200D48117A/WV-137~~
				D																											~~200046504A/WV-138~~
				D																											~~1F62748427/WV-139~~
				D																											~~201D483546/WV-140~~
17 C12 Alhydroge Max 10ugF1 10ug Mauro-V																															201D2E2A6B/WV-141
																															200A05272A/WV-142
																															2000137756/WV-143
																															1F6C7F3343/WV-144 re
																															1F7D60453F/WV-145
																															2041580443/WV-146
	D	IE	D		D	D	R	IN	G		CHAL	LE	NGE																		1F75787C78/WV-147 — DIED
																															1F20264457/WV-148
																															1F560D116D/WV-149
																															1F73671E69/WV-150
18 C12 Greer Plague Vaccine	D	IE	D	D	V	R	IN	G		CHAL	LE	NGE																			No chip - DIED
				D																											~~No chip~~
				D																											~~No chip~~
				D																											~~No chip~~
				D																											~~No chip~~
				D																											~~No chip~~
				D																											~~No chip~~
				D	D																										~~No chip~~
				D	D																										~~No chip~~
	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
19 C12 Alhydroge Max alone				D																											~~2000097B62/WV-122~~
				D																											~~1F6670384A/WV-123~~
					D	D																									~~20093B4B4F/WV-125~~
					D																										~~201E073506/WV-127~~
					D																										~~WV-129 Missing chip~~
	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	2035406407
	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	

GROUP COLOR (HAND)

RUN COLOR (INK)

For Animal Caretakers: Contact LTC Anderson, Office Ext 4933, Fax Ext 2152, Home (301) 473-5059 if you have any questions

Discard dead animals

Use scanner to check chip number of dead mice

Mark number of animals alive in each cage

re = mouse has been rechipped

File: F1-V,longterm Last update: REDACTED
Protocol: B95-01
Investigators: Anderson/Heath/Welkos/Friedlander

Background: F1-WV and F1 and V in combination have been shown to protect against challenges of CO92 and C12 with a two dose schedule (0 and 30). The long-term decay of the antibody response to the initial immunization and length of protection from a single immunization is currently not known.

Purpose: To examine the decay of the antibody response to an initial immunization, protection afford over time to an initial immunization to indicate the optimum time for the 2nd immunization for an aerosol challenge. Titers to F1 and V will be determined. Mice will be challenged with **50-100 LD_{50} CO92,** aerosol challenge.

In the below challenge groups, when protection falls to zero, the remaining groups will be booster and challenged 2 weeks post-boost.

Immunogens: EcF1s, Mauro's V, and F1-WV all essentially endotoxin free from Dr. Heath.

Mice: Swiss Webster (Hsd:ND4) female mice per group from Harlan Sprague Dawley, Inc. Indianapolis, Indiana (317) 894-7521.

Alhydrogel: 1.3%, from SuperFos. Batch # 2043, Expiration date None, 0.1857 mg of AL/dose

IMI-1000, Implantable micro identification transponders, BioMedic Data Systems, Inc., 255 W. Spring Valley Ave. Maywood, NJ 07607, Tel 201 587-8300.

Plague USP, Lot # 112FX1, Expiration Date REDACTED, Greer Laboratories, Inc. P.O Box 800 Lenoir, NC 28645-0800

Group	Treatment	Challenge Day	#of mice	
1A	10µF1+20µgMauro-V day0	14	10	serum # 6990-7039 pre challenge tissue # 10146-10160 see page 81, 85 serum # 7830-7837 day 28 PI pre challenge
1B			05	
2A	30µgF1-WV	14	10	
2B			05	
3	Plague USP	14	10	
4	Alhydrogel alone	14	10	
5A	10µF1+20µgMauro-V day0	42	10	serum # 7676-7725 pre challenge tissue # 10160-10191 day 30 PI serum # 8004-8035 day 30 PI pre challenge
5B			05	
6A	30µgF1-WV	42	10	
6B			05	
7	Plague USP	42	10	
8	Alhydrogel alone	42	10	
9A	10µF1+20µgMauro-V day0	~~93~~ 119	10	serum # 8288-8337 pre challenge serum # 8591-8621 day 28 PI tissue # 10259-10290 day 28 PI
9B			05	
10A	30µgF1-WV	~~93~~ 119	10	
10B			05	
11	Plague USP	~~93~~ 119	10	
12	Alhydrogel alone	~~93~~ 119	10	

13A	10µF1+20µgMauro-V day0	10
13B		05
14A	30µgF1-WV	10
14B		05
15	Plague USP	10
16	Alhydrogel alone	10
17A	10µF1+20µgMauro-V day0	10
17B		05
18A	30µgF1-WV	10
18B		05
19	Plague USP	10
20	Alhydrogel alone	10
	Total	250

10 serial bleeds on challenge days,

14, 42, 93, and _____

Schedule

17Oct95	Mice arrive B412
25Oct95	Mice chipped B412, Plumtree, Zimmerman
31Oct95	Mice immunized day 0, Plumtree, Zimmerman
07Nov95	Bleed 1st challenge group, day 7
14Nov95	1st challenge group, day 14, 100 LD_{50} aerosol challenge, CO92
14Nov95	Bleed serial bleed group, day 14
05Dec95	Bleed 2nd challenge group, day 35
12Dec95	2nd challenge group, day42, 100 LD_{50} aerosol challenge, CO92
12Dec95	Bleed serial bleed group, day 42
25Jan96	Bleed 3rd challenge group, 86 DAY
01Feb96	3rd challenge group, day 93, 100 LD_{50} aerosol challenge, CO92
01Feb96	Bleed serial bleed group, day 93
_____95	Bleed 4th challenge group, day
_____96	4th challenge group, day, 100 LD_{50} aerosol challenge, CO92
_____96	Bleed serial bleed group, day

Chips Numbers

22254D6722/LT-001 GP1A
22254B4164/LT-002
221D487E7E/LT-003
221D705617/LT-004
22213D0B42/LT-005
222122186F/LT-006
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2222411328/LT-018
22225C6018/LT-019
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221D4E2460/LT-021 GP3

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221D5B6077/LT-033
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221D682963/LT-036
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[redacted]/LT-245
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[redacted]/LT-247 - LOST CHIP, REPLACED 1F7E1B695F
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22254A3C0B/LT-249
221A422E06/LT-250
1F72777B7D/TESTCHIP

Exhibit GA10

REDACTED

Project: Active F1-WV, F1+V Longterm study *DAY 14 POST IMMUNIZATION*

Notebook #: 3739

Inoculum: Yersinia pestis strain, CO92 *LD50 82-108*

Route: AEROSOL | Dose: as shown below | REDACTED

Swiss Webster | Age: 7-8wks | Vendor: Harlan Sprague Dawley | Sex: female

REDACTED

Day	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	1	2	3	4	5	6	7	8	9	10	11	12	1
Day postinfection	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29

Group	Run	Head color	Comments/Chip #
1A 10ugF1+ 20ug nauro-v	CO92	*WHITE BLACK*	22254D6722/LT-001
			22254B4164/LT-002
			221D487E7E/LT-003
			221D705617/LT-004
			22213D0B42/LT-005
		DIED DURING AEROSOL RUN	222122186F/LT-006
7.0 ± 2.6			22223E1239/LT-007
		BLACK GREEN	~~2221483775/LT-008~~ *→ CHIP HANGING OUT*
MTD ± SD			221D670720/LT-009
			22213D5220/LT-010
1B 10ugF1+ 20ug nauro-v	CO92	*BLUE GREEN RED*	~~2221463FTB/LT-201~~
			~~22213F0074/LT-202~~
			~~22215A2E71/LT-203~~
			~~22223B1136/LT-204~~
			~~22215A7309/LT-205~~
		xxxxxxxx	
		xxxxxxxx	
		xxxxxxxx	
		xxxxxxxx	
		xxxxxxxx	
2A 10ug F1-VV	CO92	*RED BLACK*	~~222233166C/LT-011~~
			~~2227765159/LT-012~~
			~~221D6C477D/LT-013~~
7.0 ± 2.2			~~2222485A77/LT-014~~
			221A2B093D/LT-015
			~~221D6B7917/LT-016~~
			221D686E17/LT-017
MTD ± SD			2222411328/LT-018
		BLACK BLUE	~~22225C6018/LT-019~~
2B 10ug F1-VV	CO92	*WHITE RED*	221D630D5E/LT-020
			~~221D6B4C53/LT-206~~
			~~221D49253C/LT-207~~
			~~221D710C76/LT-208~~
			~~22273F017G/LT-209~~
			~~22217E5E6E/LT-210~~
		xxxxxxxx	
		xxxxxxxx	
		xxxxxxxx	
		xxxxxxxx	
		xxxxxxxx	
3 Plague USP Greer	CO92	*BLUE BLACK*	~~221D4E2460/LT-021~~
			~~22280F622F/LT-022~~
			221D570056/LT-023
8.7 ± 3.6			~~221D552A40/LT-024~~
			~~22223C1767/LT-025~~
		BLUE RED	~~2221534945/LT-026~~
MTD ± SD			~~22277D284D/LT-027~~
			~~221D660734/LT-028~~
			221B495727/LT-029
			2225577D72/LT-030
4 Alhydrogel alone	CO92	*GREEN BLACK*	~~221B45423B/LT-031~~
			~~221B4C8576/LT-032~~
			~~221D5B6077/LT-033~~
3.6 ± 0.7			~~2222465171/LT-034~~
			~~22277E167A/LT-035~~
		GREEN RED	~~221D682963/LT-036~~
MTD ± SD			~~222251294B/LT-037~~
			~~221B464852/LT-038~~
			~~221D605813/LT-039~~
			~~221D762A24/LT-040~~

For Animal Caretakers: Contact LTC Anderson, Office Ext 4933, Fax Ext 2152, Home (301) 473-5059 if you have any questions

Discard dead animals

Check chip number of dead mice with scanner

Mark number of animals alive in each cage

LOST after Chip # means the chip has fallen out

HEAD COLOR

BLACK RUN 6

RED RUN 7

Exhibit GA11

File: Alhydrogel concentration
Last updated: REDACTED

Anderson/Heath/Welkos/Friedlander

Background. The allowable Al content in a human vaccine is 0.85 mg/dose as determined by assay (21 CFR 610.15(1)). However, the lowest possible dose of Al should be used which maintains an adequate adjuvant response with the EcF1c and V immunogens. A dose response for Alhydrogel has not been done with a combination of F1 and V. Therefore this experiment will examine a range of concentrations of AL which will be used with a constant amount of EcF1c and V. EcF1c (60 EU/ml) and V-His tag (preparation are essentially endotoxin free. The level of endotoxin in the thrombin treated V preparation without the His tag is 49 EU/ml.

Compare the antibody response to V with F1-WV protein with and without alhydrogel. When F1 + V is used to immunize without alhydrogel, there was not antibody response to V. This will be done with F1-WV in order to determine whether F1 is contributing anything to protection with the F1-WV protein. F1-WV purified by Ni++ and ran through a Sartorius Q15 filter using 10mMtris, ph 7.6, 0.5mM EDTA + 0.5 MNaCl for elution. See Heath's note of 5-6 Dec. Endotoxin ____ EU/ml.

Mice: Swiss Webster (Hsd:ND4) female mice from Harlan Sprague Dawley, Inc. Indianapolis, Indiana (317) 894-7521.

1.3% Alhydrogel (Aluminium Hydroxide Gel Adjuvant): Al_2O_3 (1.3%) equivalent to $Al(OH)_3$ (2.0%), from SuperFos Biosector a/s, Frydeniundsvej 30, DK-2950 Vedbaek, Denmark. Batch # 2043, Expiration date None: U.S. supplier - Accurate Chemical & Scientific Corp, 300 Shames Drive, Westbury, NY 11590, Tel (516) 333-2221, Fax (516) 997-4948.

Al = 13 O = 8 H = 1, $Al(OH)_3$ = 40 molecular weight

Current procedure for adsorption of F1 and V to Alhydrogel: 1.0ml Alhydrogel brought to 7.0 ml
2% $Al(OH)_3$ = 20 mg/ml
20mg/ml)(1.0ml) = (x)(7.0ml final volume) x = 2.857 mg $Al(OH)_3$/ml
(2.857mg/ml)(0.2ml dose) = 0.57142 mg $Al(OH)_3$
AL is 0.325% of $Al(OH)_3$
0.235% of 2.857mg = 0.1857 mg of AL/0.2ml dose which the mouse receives

Current dose of Al which has been used through out the mouse experiments is 0.1857mg. Try two other doses each 75% of the former GP1 = 0.1857mg (standard amount of AL), GP2 = 0.1393mg, GP3 = 0.1045mg

IMI-1000, Implantable micro identification transponders, BioMedic Data Systems, Inc., 255 W. Spring Valley Ave. Maywood, NJ 07607, Tel 201 587-8300.

Plague USP, Lot # 1128X1, Expiration Date REDACTED, Greer Laboratories, Inc. P.O Box 800 Lenoir, NC 28645-0800,

Groups	Treatment	V-HIS Tag	Strain	#Mice
000A	30µgF1-WV, no AL	Yes	CO92	10
000B	30µgF1-WV, no AL	Yes	CO92	05
00A	30µgF1-WV+0.19Al	Yes	CO92	10
00B	30µgF1-WV+0.19Al	Yes	CO92	05
0A	10µgF1+20µgV+0.19Al	No	CO92	10
0B	10µgF1+20µgV+0.19Al	No	CO92	10

1A	10µgF1+20µgV+0.19Al	Yes	CO92		10
1B	10µgF1+20µgV+0.19Al	Yes	CO92		10
2A	10µgF1+20µgV+0.14Al	Yes	CO92		10
2B	10µgF1+20µgV+0.14Al	Yes	CO92		10
3A	10µgF1+20µgV+0.10Al	Yes	CO92		10
3B	10µgF1+20µgV+0.10Al	Yes	CO92		10
4	0.19Alhydrogel		CO92		10
5	0.19Alhydrogel		CO92		10
6	0.14Alhydrogel		CO92		10
7	0.10Alhydrogel		CO92		10
8	10µgF1+20µgV, No Alh	Yes	CO92		10
9	10µgF1+20µgV, No Alh	Yes	CO92		10
10	Plague USP (Greer)sc		CO92		10
11	Plague USP (Greer)sc		CO92		10
12	No treatment		CO92		10
13	Plague USP (Greer) im		CO92		10
				Total	210

from USAMRIID protocol

Date	Procedure
09Nov95	Arrival of mice in B412 at 7-8 weeks of age
16Nov95	Chipped mice in B412, SGT Zimmerman/Plumtree
06Dec95	Day 0, immunization Anderson/Shamblin
12Jan96	Day37, bleed prior to challenge, serum # 8045-8254 PLUMTREE, ANDERSON, SHAMBLIN
19Jan96	Day44, challenge ANDERSON, LIVINGSTON IN ANIMAL ROOM, + BLUE 1ST 3 RUNS
15Feb96	Day 28 pi, terminal bleed, spleen removal
	serum # spleen #

Chip numbers

221D682232/ALH-001 GP000A
2225353065/ALH-002
222543363A/ALH-003
2227471E04/ALH-004
2221462517/ALH-005
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2222382716/ALH-033
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2225561B32/ALH-144
2227475E7D/ALH-145

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See pages 104-107

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2227337D7F/ALH-183
22225C0D1D/ALH-184
221D4C6901/ALH-185
22277B6E20/ALH-186
2221690A60/ALH-187
222846320A/ALH-188
222161387E/ALH-189
221B30696B/ALH-190
22282E3D60/ALH-191 GP11
2221490E6D/ALH-192
2221627F79/ALH-193
2222321175/ALH-194
2221641439/ALH-195
2225443B3A/ALH-196
2222585764/ALH-197
22213B7E14/ALH-198
222160194A/ALH-199

Voir pages 104-107

REDACTEI

2221490005/ALH-200
22213D1B3C/ALH-201 GP12
2222523865/ALH-202
2221707A4B/ALH-203
221B41083C/ALH-204
222837504A/ALH-205
22273A4C62/ALH-206
22216B1128/ALH-207
221B511B39/ALH-208
2222557D5A/ALH-209
221B432713/ALH-210 GP13
221D73342A/ALH-016
221D492B54/ALH-017
2228145E46/ALH-018
221D763028/ALH-019
2221543C07/ALH-020
22225A6150/ALH-036
2221534A52/ALH-037
221D73450D/ALH-038
22276D5C6E/ALH-039
2227557710/ALH-040

Ale pages 104-107

REDACTEI

From USAMRIID notebook of Dave Heath

221B41083C/ALH-204
222837504A/ALH-205
22273A4C62/ALH-206
[illegible]6R112R/ALH-207

[redacted]

SET UP ABSORPTIONS FOR LTC ANDERSON:

GRPS 000A + 000B used F1-V that had been purified by Ni^{2+} & run through a Sartorius Q15 filter using 10 mM Tris, pH 7.6 0.5 mM EDTA + 0.5 M NaCl for elution. Buffer exchanged this using 10 mM Tris, pH 7.6 + 0.5 mM EDTA using a centricon. Did BCA assay for protein conc = 690 ug/mL and 1.5 mL total volume

so used 450 ug in 3 mL PBS = 652 ul of F1V (@ 690 ug/mL)

652 ul F1-V + 2.348 mL PBS

GRPS 00A 00B same F1-V as above @ 690 ug/mL

add 652 ul of F1-V to 428 ul ALHYDROGEL & ABSORBED FOR 2 HR @ 4°C

- spun tube @ 2000 rpm for 5 min. & removed 2 100 ul aliquots to check for protein conc. on BCA assay (see BCA from results)

- added PBS to absorbed ALH-F1V to 3 mL

PS 0A + 0B Took F1 capsule extract @ 60 EU/mL & conc. of 705 ug/mL & added 496 ul of this to 1 mL ALHYDROGEL + 137 ul of thrombin treated Vaccine (5.1 mg/mL) = 49 EU/mL

this is for 10 ug/200 ul dose of F1 + 25 ug/200 ul dose V in a final volume of 7 mL

496 ul of F1 = 350 ug 137 ul V = 700 ug V

496 ul F1 + 137 ul thrombin V + 367 ul PBS + 1 mL ALHYDROGEL

REDACTED (cont)

GRPS 1A + 1B	10 mg FX + 20 mg hV in 7 mL final volume	hV = 1.3 EU/mL
	496 uL FI (705 mg/mL) + 129 uL hV (5.14 mg/mL) + 375 uL PBS	
	+ 1 mL ALHYDROGEL, Rock OV @ 4°C	
	- Spin 2000 rpm, 5 min: remove 2 x 100 uL aliquots for BCA Assay of absorption	
	- Then added PBS to 7 mL final volume	
GRPS 2A + 2B	same as 1A + 1B above but used 750 uL ALHYDROGEL + additional 250 uL PBS	
GRPS 3A + 3B	same as 1A + 1B above but used 562.5 uL ALHYDROGEL + 437.5 uL PBS to equal 1 mL then same as 1A + 1B	
GRP 4 + 5	added 1 mL ALHYDROGEL to 1 mL PBS + rocked OV. Then added PBS to 7 mL final volume	
GRPS 6	added 750 uL ALHYDRO + 250 uL PBS + 1 mL PBS + rocked OV @ 4°C. Then added PBS to 7 mL final volume.	
GRP 7	added 562.5 uL ALHYDROGEL + 437.5 uL PBS then 1 mL PBS + rocked OV @ 4°C	
	-added PBS to 7 mL final volume	
GRP 8+9	added 496 uL FI + 129 uL hV + 1.375 mL PBS + rocked OV. Then 100.1 PBS to 7 mL final volume	

BCA assay results:

BSA 1:10	.181	.187
1:20	.079	.086
1:40	.035	.041
1:80	.012	.015

FI + hV .14 (2A+2B)

#1	#2
.100	.100

FI + hV .19 undil (1A+1B)

#1	#2
-.007	-.001

FI + hV) (3A+3B) .10

#1	#2
-.003	-.015

FI + TV .19 and 1/2 (2A+2B

#1	#2
-.003	-.002

FI-V HUMIDIFIED .19 (200A + 100B)

.004
.003

Sartorius Q15 experiment

- had 5mL of FIV @ ~ 2mg/mL + did Q15 exp. as follows:

1) equilibrated Q15 w/ 15 mL 10mM Tris (pH 7.6), 0.5 mM EDTA,
2) APPLIED 4.5mL of FIV in 10mM Tris, 0.5 mM EDTA pH 7.6 to filter (collected "FLOW THROUGH")
3) WASHED Q15 w/ 10mL Tris-EDTA equilibration buffer + collected as "Wash"
4) eluted w/ 12mL Tris, EDTA + 100 mM NaCl & collected
5) " " " " " + 200 mM NaCl " "
6) " " " " " + 300 mM NaCl " "
7) " " " " " + 400 mM NaCl " "
8) " " " " " + 500 mM NaCl " "

1.0 mL of ... 0 ... 500 mM NaCl 10mM Tris pH 7.6 each (collected)

Exhibit GA13

REDACTED		Project: F1+V alhydrogel concentrations, V w&wo His tag, F1-WV w&wo alhydrogel
Notebook #: 3739		
Inoculum: Yersinia pestis strain, CO92		
Route: aerosol		Dose: as shown below LD50 190 - 304
REDACTED	Swiss Webster	Arri REDACTED 7-8wks · Vendor: Harlan Sprague Dawley · Sex: female

	Day -	19	20	21	22	23	24	REDACTED				29	30	31	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17		
Day postinfection		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	Comments/Chip #	
Group	LD50																																
000A	CO92	l	l	l	l	l	l	l	l	l	l	l	l	l	l	l	L	L	l	l	l	l	l	l	l	l	l	l	l	l		221D682232/ALH-001	7
F1-WV		l	l	l	l	l	l	l	l	l	l	l	l	l	l	l	L	L	l	l	l	l	l	l	l	l	l	l	l	l		2225353065/ALH-002	1
No alhydr		l	l	l	Y																											~~222543363A/ALH-003~~	4
30ug	EXP -	l	l	l	l	l	l	l	l	l	l	l	l	l	l	l	L	L	l	l	l	l	l	l	l	l	l	l	l	l		2227471E04/ALH-004	3
		l	l	l	l	l	l	l	l	l	l	l	l	l	l	l	L	L	l	l	l	l	l	l	l	l	l	l	l	l		2221462517/ALH-005	5
		l	l	l	l	l	l	l	l	l	l	l	l	l	l	l	L	L	l	l	l	l	l	l	l	l	l	l	l	l		221D63634C/ALH-006	6
		l	l	l	l	l	l	l	l	l	l	l	l	l	l	l	L	L	l	l	l	l	l	l	l	l	l	l	l	l		221D645F12/ALH-007	1
		DIED DURING RUN 8																														221B2F0113/ALH-008	8
		l	l	l	l	l	l	l	l	l	l	l	l	l	l	l	L	L	l	l	l	l	l	l	l	l	l	l	l	l		222537362C/ALH-009	3
		l	l	l	l	l	l	l	l	l	l	l	l	l	l	l	L	L	l	l	l	l	l	l	l	l	l	l	l	l		221B4D572B/ALH-010	6
000B	CO92	l	l	l	l	l	l	l	l	l	l	l	l	l	l	l	L	L	l	l	l	l	l	l	l	l	l	l	l	l		221D3A1A63/ALH-011	1
F1-WV		l	l	l	l	l	l	l	l	l	l	l	l	l	l	l	L	L	l	l	l	l	l	l	l	l	l	l	l	l		2227593C63/ALH-012	3
No alhydr		l	l	l	l	l	l	l	l	l	l	l	l	l	l	l	L	L	l	l	l	l	l	l	l	l	l	l	l	l		22213E5370/ALH-013	5
30ug		l	l	l	l	l	l	l	l	l	l	l	l	l	l	l	L	L	l	l	l	l	l	l	l	l	l	l	l	l		2228111E27/ALH-014	1
		l	l	l	l	l	l	l	l	l	l	l	l	l	l	l	L	L	l	l	l	l	l	l	l	l	l	l	l	l		221B4D060F/ALH-015	
		xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx																															
		xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx																															
		xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx																															
		xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx																															
		xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx																															
00A	CO92	l	l	l	l	l	l	l	l	l	l	l	l	l	l	l	L	L	l	l	l	l	l	l	l	l	l	l	l	l		2221291C51/ALH-021	3
F1-WV		l	l	l	l	l	l	l	l	l	l	l	l	l	l	l	L	L	l	l	l	l	l	l	l	l	l	l	l	l		22217A1916/ALH-022	1
Alhydrogel		l	l	l	l	l	l	l	l	l	l	l	l	l	l	l	L	L	l	l	l	l	l	l	l	l	l	l	l	l		22215A4825/ALH-023	8
30ug		l	l	l	l	l	l	l	l	l	l	l	l	l	l	l	L	L	l	l	l	l	l	l	l	l	l	l	l	l		22213D0C4F/ALH-024	1
		l	l	l	l	l	l	l	l	l	l	l	l	l	l	l	L	L	l	l	l	l	l	l	l	l	l	l	l	l		2221661921/ALH-025	2
		l	l	l	l	l	l	l	l	l	l	l	l	l	l	l	L	L	l	l	l	l	l	l	l	l	l	l	l	l		2221501E3C/ALH-026	6
		l	l	l	l	l	l	l	l	l	l	l	l	l	l	l	L	L	l	l	l	l	l	l	l	l	l	l	l	l		221D62561C/ALH-027	9
		l	l	l	l	l	l	l	l	l	l	l	l	l	l	l	L	L	l	l	l	l	l	l	l	l	l	l	l	l		2221796313/ALH-028	5
		l	l	l	l	l	l	l	l	l	l	l	l	l	l	l	L	L	l	l	l	l	l	l	l	l	l	l	l	l		22216A4521/ALH-029	3
		DID NOT CHALLENGE																														221D5B654E/ALH-030	
00B	CO92	l	l	l	l	l	l	l	l	l	l	l	l	l	l	l	L	L	l	l	l	l	l	l	l	l	l	l	l	l		221D5B165D/ALH-031	3
F1-WV		l	l	l	l	l	l	l	l	l	l	l	l	l	l	l	L	L	l	l	l	l	l	l	l	l	l	l	l	l		2225535315/ALH-032	1
Alhydrogel		l	l	l	l	l	l	l	l	l	l	l	l	l	l	l	L	L	l	l	l	l	l	l	l	l	l	l	l	l		2222382716/ALH-033	5
30ug		l	l	l	l	l	l	l	l	l	l	l	l	l	l	l	L	L	l	l	l	l	l	l	l	l	l	l	l	l		221D64205D/ALH-034	2
		l	l	l	l	l	l	l	l	l	l	l	l	l	l	l	L	L	l	l	l	l	l	l	l	l	l	l	l	l		2221361744/ALH-035	1
		xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx																															
		xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx																															
		xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx																															
		xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx																															
		xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx																															
0A	CO92	l	l	l	l	l	l	l	l	l	l	l	l	l	l	l	L	L	l	l	l	l	l	l	l	l	l	l	l	l		221B3B756D/ALH-041	9
10F1+20V		l	l	l	l	l	l	l	l	l	l	l	l	l	l	l	L	L	l	l	l	l	l	l	l	l	l	l	l	l		2222505C62/ALH-042	1
0.19 Alh		DIED HOLDER																														221D355F0B/ALH-043	
No His tag		l	l	l	l	l	l	l	l	l	l	l	l	l	l	l	L	L	l	l	l	l	l	l	l	l	l	l	l	l		2221393824/ALH-044	1
		l	l	l	l	l	l	l	l	l	l	l	l	l	l	l	L	L	l	l	l	l	l	l	l	l	l	l	l	l		2221260562/ALH-045	6
		l	l	l	l	l	l	l	l	l	l	l	l	l	l	l	L	L	l	l	l	l	l	l	l	l	l	l	l	l		2221750728/ALH-046	8
		l	l	l	l	l	l	l	l	l	l	l	l	l	l	l	L	L	l	l	l	l	l	l	l	l	l	l	l	l		2221710C75/ALH-047	4
		l	l	l	l	l	l	l	l	l	l	l	l	l	l	l	L	L	l	l	l	l	l	l	l	l	l	l	l	l		22254A533A/ALH-048	5
		NOT CHALLENGED																														221D48425F/ALH-049	
		l	l	l	l	l	l	l	l	l	l	l	l	l	l	l	L	L	l	l	l	l	l	l	l	l	l	l	l	l		222547047F/ALH-050	2
0B	CO92	l	l	l	l	l	l	l	l	l	l	l	l	l	l	l	L	L	l	l	l	l	l	l	l	l	l	l	l	l		221B431E0B/ALH-051	4
10F1+20V		DIED IN HOLDER																														222141267F/ALH-052	-
0.19 Alh		l	l	l	l	l	l	l	l	l	l	l	l	l	l	l	L	L	l	l	l	l	l	l	l	l	l	l	l	l		2225447977/ALH-053	6
No His tag		DIED HOLDER																														22217C5904/ALH-054	-
		l	l	l	l	l	l	l	l	l	l	l	l	l	l	l	L	L	l	l	l	l	l	l	l	l	l	l	l	l		22276E4F74/ALH-055	4
		l	l	l	l	l	l	l	l	l	l	l	l	l	l	l	L	L	l	l	l	l	l	l	l	l	l	l	l	l		2222513264/ALH-056	2
		l	l	l	l	l	l	l	l	l	l	l	l	l	l	l	L	L	l	l	l	l	l	l	l	l	l	l	l	l		221B331A0B/ALH-057	1
		DID NOT CHALLENGE																														22223A2C20/ALH-058	
		l	l	l	l	l	l	l	l	l	l	l	l	l	l	l	L	L	l	l	l	l	l	l	l	l	l	l	l	l		2225301E16/ALH-059	8
		l	l	l	l	l	l	l	l	l	l	l	l	l	l	l	L	L	l	l	l	l	l	l	l	l	l	l	l	l		2222464F0C/ALH-060	5

For Animal Caretakers: Contact LTC Anderson, Office Ext 4933, Fax Ext 2152, Home (301) 473-5059 if you have any questions
Discard dead animals
Check chip number of dead mice with scanner — EXP - MOUSE CAME OUT OF HOLDER DURING CHALLENGE
Mark number of animals alive in each cage
LOST after Chip # means the chip has fallen out

REDACTED

REDACTED

REDACTED

Project: F1+V alhydrogel concentrations, V w&wo His tag, F1-WV w&wo alhydrogel

Yersinia pestis strain, CO92

Dose as shown below LD50 190-304

Weight: Arr 7-8wks Vendor: Harlan Sprague Dawley Sex: female

19	20	21	22	23	24	29	30	31	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17								
0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	Comments/Chip #	

1A	LD50																													2225525E7E/ALH-061	9
10F1+20V																														22214B6521/ALH-062	1
0.15 Alh																														2225427F32/ALH-063	4
His tag																														221B2E1A28/ALH-064	5
																														22277F2A4F/ALH-065	7
																														221B381564/ALH-066	8
																														22276C4058/ALH-067	6
																														221B365521/ALH-068	4
D	D		NOT	CHALLENGED																										2221483A1E/ALH-069	
																				DIED IN HOLDER										221B2F4049/ALH-070	
1B	CO92																																
10F1+20V		DIED			HOLDER																									2222490E7E/ALH-071	9
0.15 Alh																														22216B6702/ALH-072	
His tag																														22281D7640/ALH-073	6
																														221D74327B/ALH-074	4
																														221B36013C/ALH-075	7
																														2227177522/ALH-076	1
																														221B560D16/ALH-077	1
D	D	NOT	CHALLENGED																											222734024F/ALH-078	
																														221B463462/ALH-079	5
																														221B463656/ALH-080	8
2A	CO92																																
10F1+20V										DIED IN HOLDER																				22252D6D26/ALH-081	
0.14 Alh																														2225416D0B/ALH-082	5
His tag		DIED			HOLDER																									221B360D60/ALH-083	
																														22215C0152/ALH-084	8
																														222550400F/ALH-085	7
																														221B496E3F/ALH-086	4
																														2221353A4B/ALH-087	6
																														221D542860/ALH-088	9
NOT	CHALLENGED																													2225376005/ALH-089	
																														221B3A5805/ALH-090	4
2B	CO92																																
10F1+20V																														221D74480D/ALH-091	4
0.14 Alh																														221B4D381A/ALH-092	9
His tag																														2225315130/ALH-093	9
																														2221630F69/ALH-094	7
																														221D615D3E/ALH-095	4
																														2225574367/ALH-096	8
																														221B440F29/ALH-097	6
DIED		HOLDER																												222176302B/ALH-098	
																														22212E212C/ALH-099	7
																														22273B1603/ALH-100	5
3A	CO92																																
10F1+20V																														22274C0115/ALH-101	7
0.10 Alh																														222178537A/ALH-102	4
His tag																														2225447B43/ALH-103	6
																														2221775C19/ALH-104	5
																														2227351528/ALH-105	8
																														22271B745A/ALH-106	7
																														2221525A38/ALH-107	3
DIED		HOLDER																												2225546B51/ALH-108	
																														2225316828/ALH-109	9
																														222243260B/ALH-110	2
3	CO92																																
10F1+20V																														2221694D14/ALH-111	9
0.10 Alh																														22277D2440/ALH-112	5
His tag																														2225407A6C/ALH-113	6
																														2221445B1C/ALH-114	4
																														221D6E5550/ALH-115	1
																														2227514F27/ALH-116	3
																														22214B6D67/ALH-117	9
																														22254F0206/ALH-118	4
																														221B3B1F65/ALH-119	7
																														222543090C/ALH-120	8

r Animal Caretakers: Contact LTC Anderson, Office Ext 4933, Fax Ext 2152, Home (301) 473-5059 if you have any questions
scard dead animals
ieck chip number of dead mice with scanner
irk number of animals alive in each cage
IST after Chip # means the chip has fallen out

REDACTED

Project: F1+V alhydrogel concentrations, V w&wo His tag, F1-WV w&wo alhydrogel

Notebook #: 3739

Inoculum: Yersinia pestis strain, CO92

Route: aerosol | Dose: as shown below LD50 190-304

REDACTED Swiss Webster | Ar REDACTED 7-8wks | Vendor: Harlan Sprague Dawley | Sex: female

Group	LD50	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	Comments/Chip #
Day -		19	20	21	22	23	24	REDACTED				29	30	31	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	
4 0.19 Alh control	CO92	I	I	I	D																											~~2225402D66/ALH-121~~
		I	I	I	D																											~~2228015179/ALH-122~~
		I	I	I	D																											~~2225343914/ALH-123~~
		I	I	I	D																											~~222529444C/ALH-124~~
		I	I	I	D																											~~22271B7357/ALH-125~~
		I	I	I	D																											~~222732682C/ALH-126~~
		I	I	I	D																											~~2221501931/ALH-127~~
		I	I	I	D																											~~22196E7321/ALH-128~~
		I	I	I	D																											~~22280E5F2D/ALH-129~~
		I	I	I	D																											~~222551747C/ALH-130~~
5 0.19 Alh control	CO92	I	I	I	D																											~~2222464E2F/ALH-131~~
		I	I	I	D																											~~22272E117A/ALH-132~~
		I	I	I	D																											~~22225C6636/ALH-133~~
		I	I	I	D																											~~22254E3756/ALH-134~~
		I	I	I	D																											~~22253D1205/ALH-135~~
		I	I	I	D																											~~22216A047B/ALH-136~~
		I	I	I	D																											~~22216C346D/ALH-137~~
		DIED DURING RUN 7 9																														2225572C56/ALH-138
		I	I	I	D																											~~221B597D3A/ALH-139~~
		I	I	I	I	D																										~~221B3D2F73/ALH-140~~
6 0.14 Alh control	CO92	I	I	I	D																											~~221D6A064C/ALH-141~~
		I	I	I	D																											~~22264C574B/ALH-142~~
		I	I	I	D																											~~22224D0523/ALH-143~~
		I	I	I	D																											~~2225561B32/ALH-144~~
		I	I	I	D																											~~2227475E7D/ALH-145~~
		I	I	I	D																											~~2221702733/ALH-146~~
		I	I	I	D																											~~221D504A22/ALH-147~~
		I	I	I	D																											~~221B330124/ALH-148~~
		I	I	I	I	D																										~~22215C754C/ALH-149~~
		I	I	I	D																											~~22253B326D/ALH-150~~
7 0.10 Alh control	CO92	I	I	I	D																											~~2222586C48/ALH-151~~
		I	I	I	D																											~~222232667C/ALH-152~~
		I	I	I	D																											~~2228452429/ALH-153~~
		I	I	I	D																											~~2222306C69/ALH-154~~
		I	I	I	D																											~~222836351D/ALH-155~~
		I	I	I	D																											~~222779775C/ALH-156~~
OK A EXP	LIVE →	DEAD HEADER																														~~221B377621/ALH-157~~
		I	I	I	D																											~~2222544F07/ALH-158~~
		I	I	I	D																											~~2221734C6B/ALH-159~~
		I	I	I	D																											~~2222442E32/ALH-160~~
8 10F1+20V No Alh His tag	CO92	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I		2222575041/ALH-161
		I	I	I	I	D																										~~2222511267/ALH-162~~
		I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I		2221516F0F/ALH-163
		NOT CHALLENGED																														221B2F761A/ALH-164
		I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I		22276B7F11/ALH-165
		I	I	I	I	D																										~~2222370F7B/ALH-166~~
		I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I		222164005D/ALH-167
		I	I	I	I	I	I	D																								~~221D705734/ALH-168~~
		I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I		221B465E6A/ALH-169
		I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I		22224C3E1B/ALH-170
9 10F1+20V No Alh His tag	CO92	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I		221D592473/ALH-171
		I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I		22280E6F44/ALH-172
		I	I	I	I	D																										~~22223D0B51/ALH-173~~
		I	I	I	I	I	D																									~~2225376F4E/ALH-174~~
		NOT CHALLENGED																														2221704167/ALH-175
		I	I	I	I	D																										~~22224D1769/ALH-176~~
		I	I	I	I	D																										~~2225312134/ALH-177~~
		I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I		221D611735/ALH-178
		I	I	I	I	D																										~~222245080B/ALH-179~~
		I	I	I	I	D																										~~221B39427A/ALH-180~~

For Animal Caretakers: Contact LTC Anderson, Office Ext 4933, Fax Ext 2152, Home (301) 473-5059 if you have any questions

Discard dead animals

Check chip number of dead mice with scanner EXP - NOT SURE MOUSE RESUSCITATION FOR EXPOSURE

Mark number of animals alive in each cage

LOST after Chip # means the chip has fallen out

REDACTED

Project: F1+V alhydrogel concentrations, V w&wo His tag, F1-WV w&wo alhydrogel

Notebook #: 3739

Inoculum: Yersinia pestis strain, CO92

Route: aerosol

Dose: as shown below LD50 191-304

REDACTED Swiss Webster A REDACTED at 7-8wks Vendor: Harlan Sprague Dawley Sex: female

Day -	19	20	21	22	23	2																									
Day post injection	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	Comments/Chip #
Group	LD50																														
10 Plague (Greer) s.c. 1128X1	CO92																														2222490F5D/ALH-181 5
																															22271D2B7D/ALH-182 7
				D																											~~2227337D7F/ALH-183~~ 4
																															22225C0D1D/ALH-184 3
	DIED - NOT CHALLENGED																														221D4C6901/ALH-185
					D																										~~22277B6E20/ALH-186~~ 5
					D																										~~2221690A60/ALH-187~~ 4
	DIED NOT CHALLENGED																														222846320A/ALH-188
									D																						222161387E/ALH-189 3
								D																							~~221B30696B/ALH-190~~ 6
11 Plague (Greer) s.c. 1128X1	CO92								D																						~~22282E3D60/ALH-191~~ 3
					D																										~~2221490E6D/ALH-192~~ 9
						D																									~~2221627E79/ALH-193~~ 3
								D																							~~2222321175/ALH-194~~ 6
	DIED NOT CHALLENGED																														2221641439/ALH-195
										D																					2225443B3A/ALH-196 5
											D																				2222585764/ALH-197 4
												D																			22213B7E14/ALH-198 5
	DIED IN HOLDER - MOUSE CANNIBAL																														222160194A/ALH-199 7
																															2221490005/ALH-200 8
12 No Treatment	CO92			D																											~~22213D1B3C/ALH-201~~ 6
				D																											~~2222523865/ALH-202~~ 5
				D																											~~2221707A4B/ALH-203~~ 3
				1	D																										~~221B41083C/ALH-204~~ 8
				D																											~~222837504A/ALH-205~~ 5
				D																											~~22273A4C62/ALH-206~~ 7
				D																											22216B1128/ALH-207 6
				D																											~~221B511B39/ALH-208~~ 3
				D																											~~2222557D5A/ALH-209~~ 4
				D																											~~221B432713/ALH-210~~ 7
13 Plague (Greer) i.m. 1128X1	CO92																														221D73342A/ALH-016 8
																															221D492B54/ALH-017 5
																															2228145E46/ALH-018 4
																															221D763028/ALH-019 7
																															2221543C07/ALH-020 3
									D																						22225A6150/ALH-036 6
																															2221534A52/ALH-037 6
	DIED AIRLINE ROW 7																														221D73450D/ALH-038 7
																															22276D5C6E/ALH-039 3
																															2227557710/ALH-040 6
	CO92																														
	CO92																														

For Animal Caretakers: Contact LTC Anderson, Office Ext 4933, Fax Ext 2152, Home (301) 473-5059 if you have any questions
Discard dead animals
Check chip number of dead mice with scanner
Mark number of animals alive in each cage
LOST after Chip # means the chip has fallen out

liv mice 88

ELISA Summary: Protocol: F1-V Long Term (Day 7)

V ELISA DATE: 30 JAN 96 F1 ELISA DATE: 25 JAN 96

Plate	Serum	Group	Treatment	V TITER	V Geomean	F1 TITER	F1 Geomean
1A	6990	GP1A10ugF1	+20ugMaurV	320	485	2,560	2,681
1B	6991	GP1A10ugF1	+20ugMaurV	640		1,280	
2A	6992	GP1A10ugF1	+20ugMaurV	2,580		10,240	
2B	6993	GP1A10ugF1	+20ugMaurV	640		2,560	
3A	6994	GP1A10ugF1	+20ugMaurV	640		2,560	
3B	6995	GP1A10ugF1	+20ugMaurV	320		2,560	
4A	6996	GP1A10ugF1	+20ugMaurV	320		2,560	
4B	6997	GP1A10ugF1	+20ugMaurV	320		2,560	
5A	6998	GP1A10ugF1	+20ugMaurV	640		5,120	
5B	6999	GP1A10ugF1	+20ugMaurV	1,280		5,120	
21A	7030	GP1B10ugF1	+20ugMaurV	320		1,280	
21B	7031	GP1B10ugF1	+20ugMaurV	320		320	
22A	7032	GP1B10ugF1	+20ugMaurV	320		5,120	
22B	7033	GP1B10ugF1	+20ugMaurV	320		2,560	
23A	7034	GP1B10ugF1	+20ugMaurV	320		5,120	
6A	7000	GP2A	30ugF1-WV	320	557	2,560	3,225
6B	7001	GP2A	30ugF1-WV	640		1,280	
7A	7002	GP2A	30ugF1-WV	640		5,120	
7B	7003	GP2A	30ugF1-WV	2,560		2,560	
8A	7004	GP2A	30ugF1-WV	640		5,120	
8B	7005	GP2A	30ugF1-WV	640		1,280	
9A	7006	GP2A	30ugF1-WV	320		2,560	
9B	7007	GP2A	30ugF1-WV	320		5,120	
10A	7008	GP2A	30ugF1-WV	1,280		5,120	
10B	7009	GP2A	30ugF1-WV	640		5,120	
23B	7035	GP2B	30ugF1-WV	640		10,240	
24A	7036	GP2B	30ugF1-WV	320		5,120	
24B	7037	GP2B	30ugF1-WV	320		10,240	
25A	7038	GP2B	30ugF1-WV	320		2,560	
25B	7039	GP2B	30ugF1-WV	640		320	
11A	7010	GP3	PlagueUSP	320	343	2,560	2,389
11B	7011	GP3	PlagueUSP	320		5,120	
12A	7012	GP3	PlagueUSP	320		2,560	
12B	7013	GP3	PlagueUSP	320		640	
13A	7014	GP3	PlagueUSP	320		5,120	
13B	7015	GP3	PlagueUSP	640		640	
14A	7016	GP3	PlagueUSP	320		2,560	
14B	7017	GP3	PlagueUSP	320		2,560	
15A	7018	GP3	PlagueUSP	320		2,560	
15B	7019	GP3	PlagueUSP	320		5,120	
16A	7020	GP4	alhydro alone	320	343	320	320
16B	7021	GP4	alhydro alone	320		320	
17A	7022	GP4	alhydro alone	320		320	
17B	7023	GP4	alhydro alone	320		320	
18A	7024	GP4	alhydro alone	320		320	
18B	7025	GP4	alhydro alone	320		320	
19A	7026	GP4	alhydro alone	320		320	
19B	7027	GP4	alhydro alone	640		320	
20A	7028	GP4	alhydro alone	320		320	
20B	7029	GP4	alhydro alone	320		320	
33B	F1/V	POOL (+ CONTROL)		327,680		81,920	

Protocol: F1-V Long Term (Day 35)

V ELISA DATE: 7 FEB 96 F1 ELISA DATE: 6 FEB 96

Plate	Serum	Group	Treatment	V TITER	V Geomean	F1 TITER	F1 Geomean
1A	7676	GP5A10ugF1	20ugVALH	1,310,720	1,040,319	40,960	25,803
1B	7677	GP5A10ugF1	20ugVALH	1,310,720		40,960	
2A	7678	GP5A10ugF1	20ugVALH	1,310,720		40,960	
2B	7679	GP5A10ugF1	20ugVALH	1,310,720		40,960	
3A	7680	GP5A10ugF1	20ugVALH	1,310,720		20,480	
3B	7681	GP5A10ugF1	20ugVALH	327,680		40,960	
4A	7682	GP5A10ugF1	20ugVALH	1,310,720		20,480	
4B	7683	GP5A10ugF1	20ugVALH	1,310,720		20,480	
5A	7684	GP5A10ugF1	20ugVALH	1,310,720		40,960	
5B	7685	GP5A10ugF1	20ugVALH	1,310,720		81,920	
6A	7686	GP5B10ugF1	20ugVALH	1,310,720		10,240	
6B	7687	GP5B10ugF1	20ugVALH	1,310,720		10,240	
7A	7688	GP5B10ugF1	20ugVALH	1,310,720		20,480	
7B	7689	GP5B10ugF1	20ugVALH	1,310,720		10,240	
8A	7690	GP5B10ugF1	20ugVALH	163,840		20,480	
8B	7691	GP6A ALH+	30ugF1-WV	655,360	948,482	81,920	9,336
9A	7692	GP6A ALH+	30ugF1-WV	1,310,720		5,120	
9B	7693	GP6A ALH+	30ugF1-WV	655,360		20,480	
10A	7694	GP6A ALH+	30ugF1-WV	1,310,720		10,240	
10B	7695	GP6A ALH+	30ugF1-WV	1,310,720		10,240	
11A	7696	GP6A ALH+	30ugF1-WV	163,840		2,560	
11B	7697	GP6A ALH+	30ugF1-WV	655,360		2,560	
12A	7698	GP6A ALH+	30ugF1-WV	1,310,720		20,480	
12B	7699	GP6A ALH+	30ugF1-WV	655,360		20,480	
13A	7700	GP6A ALH+	30ugF1-WV	1,310,720		5,120	
13B	7701	GP6B ALH+	30ugF1-WV	1,310,720		20,480	
14A	7702	GP6B ALH+	30ugF1-WV	1,310,720		20,480	
14B	7703	GP6B ALH+	30ugF1-WV	1,310,720		2,560	
15A	7704	GP6B ALH+	30ugF1-WV	1,310,720		10,240	
15B	7705	GP6B ALH+	30ugF1-WV	1,310,720		2,560	
16A	7706	GP7	Plague USP	320	735	320	4,777
16B	7707	GP7	Plague USP	1,280		20,480	
17A	7708	GP7	Plague USP	640		10,240	
17B	7709	GP7	Plague USP	640		20,480	
18A	7710	GP7	Plague USP	1,280		1,280	
18B	7711	GP7	Plague USP	640		1,280	
19A	7712	GP7	Plague USP	640		5,120	
19B	7713	GP7	Plague USP	320		2,560	
20A	7714	GP7	Plague USP	1,280		5,120	
20B	7715	GP7	Plague USP	1,280		40,960	
21A	7716	GP8	Alhydrogel	640	485	320	320
21B	7717	GP8	Alhydrogel	640		320	
22A	7718	GP8	Alhydrogel	320		320	
22B	7719	GP8	Alhydrogel	640		320	
23A	7720	GP8	Alhydrogel	640		320	
23B	7721	GP8	Alhydrogel	320		320	
24A	7722	GP8	Alhydrogel	320		320	
24B	7723	GP8	Alhydrogel	1,280		320	
25A	7724	GP8	Alhydrogel	320		320	
25B	7725	GP8	Alhydrogel	320		320	
33B	F1/V	POOL (+ CONTROL)		655,360		81,920	

Mouse weights for aerosol run of 23Feb96			
24.9			
30.9	average wt (gm)	29.98	
32.5	Cage 9A		
32.4			
29.5			
28.8			
28.2			
30.1			
34.3			
28.2			

Ig Subclass ELISA response to vaccination with *Yersinia pestis* V and F1 antigens (continued)

Plate setup:

Same as 21 FEB 96, except test samples (6) for V antigen only.

Procedure:

Same as 21 FEB 96, except:

- Second antibodies (goat anti-mouse IgG1, IgG2a, and IgG2b) diluted 1:3000 (decreased from 1:2000).
- Conjugate dilution decreased to 1:3000 (from 1:2000).

Results: see separate pages.

see page 137 redated 21 FEB 96
BALB-V day 56 postimmunization

Sample	Treatment	IgG(H+L)	IgG1	IgG2a	IgG2b
2041	Alhydrogel	320	320	320	640
2042	Alhydrogel	320	320	320	320
2043	Alhydrogel	320	320	320	320
2044	Alhydrogel	320	320	320	320
2045	Alhydrogel	320	320	320	320
2046	Alhydrogel	320	320	320	320
2047	Alhydrogel	320	320	320	320
2048	Alhydrogel	320	320	320	320
2049	Alhydrogel	320	320	320	320
2061	Alh+V	640,000	655,360	20,480	81,920
2062	Alh+V	320,000	1,310,720	81,920	10,960
2063	Alh+V	640,000	1,310,720	40,960	81,920
2064	Alh+V	320,000	655,360	40,960	40,960
2065	Alh+V	640,000	655,360	81,920	10,960
2066	Alh+V	640,000	327,680	40,960	20,480
2067	Alh+V	1,280,000	1,310,720	81,920	81,920
2068	Alh+V	1,280,000	1,310,720	40,960	163,840
2069	Alh+V	640,000	1,310,720	20,480	81,920

On mean, all samples 1640

see page 75

AEROSOL EXPOSURE SHEET

Aerosol exposure #: 56-01cH | Location: AP-4 H | Date: 23 Feb 96
Aerosol Operator: SPC Holmes | Agent: Plague (CO92)
Pre-operational Check Performed: ✓ | Dry T: 71 | Wet T: 63 | Rel. Hum.: 63% | Protocol #:
Exposure System Type/#: Nose Only | P.I.: COL Byrne
System Flow Rate: 12.0 LPM | LTC Anderson
Collison #: A
Panel #: 3
Electronic Flow Meter #: F6979
Timer Check: ✓ Pre ✓ Post
Start time 0930 1402
End time 0935 1407

Run #	Animal #	Animal Species	Start T Dry	Start T Wet	Start T Rel. Hum.	5 min T Dry	5 min T Wet	5 min T Rel. Hum.	Start Time	AGI #	Comments
1	25	Mice	71	63	63%	71	67	80%	1001	52	
2	25	Mice	73	63	58%	73	68	78.5%	1030	1163	* Cannot Lower Humidity, because all air bubbles to second air am turned off. (But)
3	25	mice	73	63	58%	73	68	78.5%	1054	361	
4	25	mice	73	63	58%	73	68	78.5%	1119	26	
5	23	Mice	74	63	55%	74	66	68%	1152	360	
6	25	Mice	75	64	57%	75	68	70%	1222	72	
7	25	Mice	75	63	51%	74	66	68%	1321	5	* One mouse dead on arrival
									B		23 Feb 96

Antibiotic Treatment / Vaccine Challenge

Date: 23-Feb

PI: COL Byrne / LTC Anderson
Agent: Plague
Strain: CO92

Animal Model: Mouse
Strain: Swiss Webster
Wt: (Ave.) : 20g / 29.98g

CO92 LD50=2.1E+04

for runs 6 & 7, Anderson

Sex: female

Run #	AGI/ml	AGI	cfu/l aerosol	MV	Inhaled Dose cfu	LD50s	
1	3.50E+07	3.50E+08	5.83E+06	0.02	1.17E+06	55.56	CO92
2	3.80E+07	3.80E+08	6.33E+06	0.02	1.27E+06	60.32	CO92
3	5.10E+07	5.10E+08	8.50E+06	0.02	1.70E+06	80.95	CO92
4	4.00E+07	4.00E+08	6.67E+06	0.02	1.33E+06	63.49	CO92
5	8.10E+06	8.10E+07	1.35E+06	0.02	2.70E+05	12.86	CO92
6	1.70E+07	1.70E+08	2.83E+06	0.027	7.65E+05	36.43	CO92
7	1.60E+07	1.60E+08	2.67E+06	0.027	7.20E+05	34.29	CO92

Date: 23Feb96 | Project: Active F1-WV, F1+V Longterm study Day 119 Postimmunization
Notebook #: 3739
Inoculum: Yersinia pestis strain, CO92
Route: aerosol | Dose: as shown below *LD50: 34-36*
Animal strain: Swiss Webster | Arrival 10/17/95 at 7-8wks | Vendor: Harlan Sprague Dawley | Sex: female

Month - Feb Day -			23	24	25	26	27	28	29	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	
Day postinfection			0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	Comments/Chip #
Group	LD50																																
9A 10ugF1+ 20ug mauro-V	CO92 *MTD ES.0 13.1 E1.0* *WHITE*	*BLACK*	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I		221D54560C/LT-081
			I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I		2221523113/LT-082
			I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I		221D4E207A/LT-083
			I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I		2225395749/LT-084
			I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I		2221340347/LT-085
			I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I		2221774E53/LT-086
			I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I		2222576612/LT-087
	BLACK GREEN		I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I		22224E6F58/LT-088
			I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I		221D563E57/LT-089
		RED	*DIED DURING CHALLENGE*																														22212D0F34/LT-090
9B 10ugF1+ 20ug mauro-V	CO92 *BLUE GREEN*	*RED*	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I		221D681542/LT-221
			I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I		22280E312B/LT-222
			I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I		221D584D78/LT-223
			I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I		221D4C4067/LT-224
			I	I	I	I	I	I	I	I	I	I	I	I	D																		~~22212A2966/LT-225~~
			xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx																														
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			xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx																														
10A 30ugF1-WV	CO92 *RED*	*BLACK*	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I		221D62392D/LT-091
			I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I		2222411F74/LT-092
			I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I		221D4D6C2C/LT-093
			I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I		221D554048/LT-094
			I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I		2221473A36/LT-095
			I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I		2221240C60/LT-096
			I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I		22214F1224/LT-097
	BLACK BLUE		I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I		2221425212/LT-098
			I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I		22223C390C/LT-099
		RED	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I		2221414605/LT-100
10B 30ugF1-WV	CO92 *WHITE*	*RED*	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I		222124374C/LT-226
			I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I		2222586303/LT-227
			I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I		2222371808/LT-228
			I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I		2222443D5B/LT-229
			I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I		22213B707C/LT-230
			xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx																														
			xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx																														
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			xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx																														
11 Plague USP Greer 1128X1	CO92 *MTD LD50 7.5E1.9* *BLUE*	*BLACK*	I	I	I	I	I	I	I	D																							~~221D6F1230/LT-101~~
			I	I	I	I	I	I	I	I	I	I	D																				2221305178/LT-102
			I	I	I	I	I	I	I	I	D																						~~2221770E09/LT-103~~
			I	I	I	I	I	I	D																								~~221D403C64/LT-104~~
			I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I		2222355935/LT-105
		RED	I	I	I	I	I	I	I	I	D																						~~221D480305/LT-106~~
			I	I	I	I	I	I	I	I	D																						~~2221633E37/LT-107~~
			I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I		22254A6B01/LT-108
			I	I	I	I	D																										~~222147520A/LT-109~~
			I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I		2221760056/LT-110
12 Alhydrogel alone	CO92 *MTD ES0 3.1E0.3* *GREEN*	*BLACK*	I	I	I	D																											~~222240T108/LT-111~~
			I	I	I	D																											~~2223422877/LT-112~~
			I	I	I	D																											~~2227610A1C/LT-113 lost~~
			I	I	I	D																											~~221D686D00/LT-114~~
			I	I	I	D																											~~221D703E2B/LT-115~~
		RED	I	I	I	D																											~~221D871449/LT-116~~
			I	I	I	D																											~~22225C0824/LT-117~~
			I	I	I	D																											~~2221586D78/LT-118~~
			I	I	I	D																											~~22214C0710/LT-119~~
			I	I	I	D																											~~221D753C07/LT-120~~

For Animal Caretakers: Contact LTC Anderson, Office Ext 4933, Fax Ext 2152, Home (301) 473-5059 if you have any questions
Discard dead animals
Check chip number of dead mice with scanner
Mark number of animals alive in each cage
LOST after Chip # means the chip has fallen out

SGT LIVINGSTON LOADED

MISAD COLOR
BLACK - AEROSOL RUN 6
RED - AEROSOL RUN 7

[illegible]

Exhibit GA15

ELISA V Summary 3 APR 96

PROTOCOL: V LONGTERM — V antigen TITER

Plate	Serum	Group	Treatment	Bleed	IgG(H+L)
1A	8288	GP9A	F1+V	+DAY86	81,920
1B	8289	GP9A	F1+V	+DAY86	327,680
2A	8290	GP9A	F1+V	+DAY86	40,960
2B	8291	GP9A	F1+V	+DAY86	NO SERUM
3A	8292	GP9A	F1+V	+DAY86	163,840
3B	8293	GP9A	F1+V	+DAY86	327,680
4A	8294	GP9A	F1+V	+DAY86	327,680
4B	8295	GP9A	F1+V	+DAY86	655,360
5A	8296	GP9A	F1+V	+DAY86	40,960
5B	8297	GP9A	F1+V	+DAY86	655,360
6A	8298	GP9B	F1+V	+DAY86	327,680
6B	8299	GP9B	F1+V	+DAY86	327,680
7A	8300	GP9B	F1+V	+DAY86	81,920
7B	8301	GP9B	F1+V	+DAY86	655,360
8A	8302	GP9B	F1+V	+DAY86	327,680
				Geomean	220,512
8B	8303	GP10A	F1-WV	+DAY86	163,840
9A	8304	GP10A	F1-WV	+DAY86	163,840
9B	8305	GP10A	F1-WV	+DAY86	163,840
10A	8306	GP10A	F1-WV	+DAY86	163,840
10B	8307	GP10A	F1-WV	+DAY86	163,840
11A	8308	GP10A	F1-WV	+DAY86	81,920
11B	8309	GP10A	F1-WV	+DAY86	163,840
12A	8310	GP10A	F1-WV	+DAY86	163,840
12B	8311	GP10A	F1-WV	+DAY86	163,840
13A	8312	GP10A	F1-WV	+DAY86	163,840
13B	8313	GP10B	F1-WV	+DAY86	81,920
14A	8314	GP10B	F1-WV	+DAY86	327,680
14B	8315	GP10B	F1-WV	+DAY86	327,680
15A	8316	GP10B	F1-WV	+DAY86	163,840
15B	8317	GP10B	F1-WV	+DAY86	327,680
				Geomean	171,589
16A	8318	GP11	PLAGUE USP	+DAY86	1,280
16B	8319	GP11	PLAGUE USP	+DAY86	640
17A	8320	GP11	PLAGUE USP	+DAY86	1,280
17B	8321	GP11	PLAGUE USP	+DAY86	640
18A	8322	GP11	PLAGUE USP	+DAY86	1,280
18B	8323	GP11	PLAGUE USP	+DAY86	1,280
19A	8324	GP11	PLAGUE USP	+DAY86	1,280
19B	8325	GP11	PLAGUE USP	+DAY86	640
20A	8326	GP11	PLAGUE USP	+DAY86	640
20B	8327	GP11	PLAGUE USP	+DAY86	1,280
				Geomean	970
21A	8328	GP12	ALHYDRO ALONE	+DAY86	640
21B	8329	GP12	ALHYDRO ALONE	+DAY86	640
22A	8331	GP12	ALHYDRO ALONE	+DAY86	640
22B	8333	GP12	ALHYDRO ALONE	+DAY86	1,280
23A	8335	GP12	ALHYDRO ALONE	+DAY86	1,280
23B	8336	GP12	ALHYDRO ALONE	+DAY86	1,280
24A	8337	GP12	ALHYDRO ALONE	+DAY86	640
				Geomean	861
24B	F1/V POOL				655,360

ELISA F1 Summary

5 APR 96

PROTOCOL: V/F1 LONGTERM — F1 antigen TITER

Plate	Serum	Group	Treatment	Bleed	IgG(H+L)
1A	8288	GP9A	F1+V	+DAY86	INSUFFICIENT SERUM
1B	8289	GP9A	F1+V	+DAY86	20,480
2A	8290	GP9A	F1+V	+DAY86	INSUFFICIENT SERUM
	8291	GP9A	F1+V	+DAY86	NO SERUM
2B	8292	GP9A	F1+V	+DAY86	40,960
3A	8293	GP9A	F1+V	+DAY86	2,560
3B	8294	GP9A	F1+V	+DAY86	20,480
4A	8295	GP9A	F1+V	+DAY86	5,120
4B	8296	GP9A	F1+V	+DAY86	20,480
5A	8297	GP9A	F1+V	+DAY86	81,920
5B	8298	GP9B	F1+V	+DAY86	40,960
6A	8299	GP9B	F1+V	+DAY86	2,560
6B	8300	GP9B	F1+V	+DAY86	20,480
7A	8301	GP9B	F1+V	+DAY86	40,960
7B	8302	GP9B	F1+V	+DAY86	5,120
				Geomean	15,343
8A	8303	GP10A	F1-WV	+DAY86	5,120
8B	8304	GP10A	F1-WV	+DAY86	20,480
9A	8305	GP10A	F1-WV	+DAY86	1,280
9B	8306	GP10A	F1-WV	+DAY86	10,240
10A	8307	GP10A	F1-WV	+DAY86	10,240
10B	8308	GP10A	F1-WV	+DAY86	2,560
11A	8309	GP10A	F1-WV	+DAY86	2,560
11B	8310	GP10A	F1-WV	+DAY86	5,120
12A	8311	GP10A	F1-WV	+DAY86	1,280
12B	8312	GP10A	F1-WV	+DAY86	5,120
13A	8313	GP10B	F1-WV	+DAY86	1,280
13B	8314	GP10B	F1-WV	+DAY86	2,560
14A	8315	GP10B	F1-WV	+DAY86	5,120
14B	8316	GP10B	F1-WV	+DAY86	5,120
15A	8317	GP10B	F1-WV	+DAY86	10,240
				Geomean	4,256
15B	8318	GP11	PLAGUE USP	+DAY86	5,120
16A	8319	GP11	PLAGUE USP	+DAY86	5,120
16B	8320	GP11	PLAGUE USP	+DAY86	10,240
17A	8321	GP11	PLAGUE USP	+DAY86	10,240
17B	8322	GP11	PLAGUE USP	+DAY86	10,240
18A	8323	GP11	PLAGUE USP	+DAY86	5,120
18B	8324	GP11	PLAGUE USP	+DAY86	10,240
19A	8325	GP11	PLAGUE USP	+DAY86	20,480
19B	8326	GP11	PLAGUE USP	+DAY86	5,120
20A	8327	GP11	PLAGUE USP	+DAY86	10,240
				Geomean	8,317
20B	8328	GP12	ALHYDRO ALONE	+DAY86	320
21A	8329	GP12	ALHYDRO ALONE	+DAY86	320
21B	8331	GP12	ALHYDRO ALONE	+DAY86	320
22A	8333	GP12	ALHYDRO ALONE	+DAY86	320
22B	8335	GP12	ALHYDRO ALONE	+DAY86	320
23A	8336	GP12	ALHYDRO ALONE	+DAY86	320
23B	8337	GP12	ALHYDRO ALONE	+DAY86	320
				Geomean	320
24A	F1/V POOL				40,960

500ml, undiluted

9APR96

Hbt No: 1	Hbt No: 2	Hbt No: 3	Hbt No: 4	Hbt No: 5	Hbt No: 6	Hbt No: 7	Hbt No: 8	Hbt No: 9	Hbt No: 10
1	1	1	1	1	1	1	1	1	1
2a 2b	2a 2b	2a 2b	2a 2b	2a 2b	2a 2b	2a 2b	2a 2b	2a 2b	2a 2b
3	3	3	3	3	3	3	3	3	3
A	A	A	A	A	A	A	A	A	A
E	E	E	E	E	E	E	E	E	E
M	M	M	M	M	M	M	M	M	M
κ	κ	κ	κ	κ	κ	κ	κ	κ	κ
λ	λ	λ	λ	λ	λ	λ	λ	λ	λ

On reverse 1 sample, all < 640 nm on 2 May 96

MAb V-YP 7F5-1-1, SUBCLONES 1-10

Mean time-to-death (MTD)

Challenge group	1	2	3	4	5	6	7	8	9	10	MTD	+/- Stdev	Std error	Group
Subcutaneous:														
1 alhydrogel alone, days 0, 30, sc														
C12 100	5	6	6	7	7	8	8	8	10	11	7.6	1.837873	0.61262	1
2 alhydrogel + 13.6 µg F1-WV fusion protein day 0, 30, sc														
C12 100	28	28	28	28	28	28	28	28	28	28	28	0	0	2
3 alhydrogel + 10 µg Mauro-V urea, days 0, 30, sc														
C12 Max	6	28	28	28	28	28	28	28	28	28	25.8	6.957011	2.319	3
4 alhydrogel + 13.6 µg F1-WV fusion protein day 0, 30, sc														
C12 Max	6	28	28	28	28	28	28	28	28	28	25.8	6.957011	2.319	4
5 alhydrogel + 27.2 µg F1-WV fusion protein day 0, 30, sc														
C12 Max	28	28	28	28	28	28	28	28	28	28	28	0	0	5
6 alhydrogel alone, days 0, 30, sc														
C12 Max	3	3	3	3	3	3	3	5	5	5	3.6	0.966092	0.32203	6
7 alhydrogel + 27.2 µg F1-WV fusion protein day 0, 30, sc														
CO92 100	28	28	28	28	28	28	28	28	28	28	28	0	0	7
8 alhydrogel alone, days 0, 30, sc														
CO92 100	3	3	4	4	4	4	6	6	7	7	4.8	1.549193	0.5164	8
Aerosol:														
9 alhydrogel alone, days 0, 30, sc														
C12 50	3	3	3	3	3	3	3	3	3		3	0	0	9
10 alhydrogel + 13.6 µg F1-WV fusion protein day 0, 30, sc														
C12 50	28	28	28	28	28	28	28	28	28	28	28	0	0	10
11 alhydrogel + 10 µg Mauro-V urea, days 0, 30, sc														
C12 Max	5	28	7	28	28	28	28	28	28	28	23.6	9.287985	3.096	11
12 alhydrogel + 13.6 µg F1-WV fusion protein day 0, 30, sc														
C12 Max	28	28	28	28	28	28	28	28	28	28	28	0	0	12

Exhibit GA16

Challenge group	1	2	3	4	5	6	7	8	9	10	MTD	MTD +/- Stdev	Std error	
13 alhydrogel + 27.2 µg F1-WV fusion protein day 0, 30, sc														
C12 Max	28	28	28	28	28	28	28	28	28	28	28	0	0	13
14 alhydrogel alone, days 0, 30, sc														
C12 Max	3	3	3	3	3	3	3	3	4		3.11111	0.333333	0.11785	14
15 alhydrogel + 13.6 µg F1-WV fusion protein day 0, 30, sc														
CO92 100	28	28	28	28	28	28	28	28	28	28	28	0	0	15
16 alhydrogel alone, days 0, 30, sc														
CO92 100	28	4	3	3	3	3	3	3	3	3	5.6	7.87683	2.62561	16
17 alhydrogel + 10 µg F1 + 10 µg Mauro's V , days 0, 30, sc														
C12 Max	28	28	28	28	28	28	28	28	28		28	0	0	17
18 Greer plague vaccine, days 0, 30, sc														
C12 Max	4	4	3	3	3	3	3	3			3.25	0.46291	0.17496	18
19 alhydrogel alone, days 0, 30, sc														
C12 Max	3	3	4	4	5						3.8	0.83666	0.41833	19

Exhibit GA17

Date: 28Jun96 | Project: Longterm F1-V, F1+V Challenge, Day 216

Notebook #: 3739

Inoculum: Yersinia pestis strain, CO92

Route: aerosol | Dose: as shown below

Animal strain: Swiss Webster | Arrival 10/17/95 at 7-8wks | Vendor: Harlan Sprague Dawley | Sex: female

Group	LD50	Color	Color	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	Comments/Chip #
Month - Jun Day -				28	29	30	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	
GP13A 10ug F1 + 20 ug V Alhydrogel	CO92	WHITE	WHITE	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	L	L	I	I	I	I	I	I	I	I		2227712235/LT-121
				I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	L	L	I	I	I	I	I	I	I	I		222241257B/LT-122
				I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	L	L	I	I	I	I	I	I	I	I		22224F1419/LT-123
				I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	L	L	I	I	I	I	I	I	I	I		221D744B1A/LT-124
				I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	L	L	I	I	I	I	I	I	I	I		22224E7477/LT-125
				I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	L	L	I	I	I	I	I	I	I	I		22224C4154/LT-126
				I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	L	L	I	I	I	I	I	I	I	I		221D66020D/LT-127
				I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	L	L	I	I	I	I	I	I	I	I		2221427332/LT-128
		WHITE	BLACK	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	L	L	I	I	I	I	I	I	I	I		221D730F06/LT-129
				I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	L	L	I	I	I	I	I	I	I	I		221D691F24/LT-130
GP13B 10ug F1 + 20 ug V Alhydrogel	CO92	RED	BLACK	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	L	L	I	I	I	I	I	I	I			221D737744/LT-231
				DIED — DURING CHALLENGE																														2221313035/LT-232
				I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	L	L	I	I	I	I	I	I	I	I		2222392702/LT-233
				I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	L	L	I	I	I	I	I	I	I	I		221D73716A/LT-234
				DIED — DURING CHALLENGE																														221D527D35/LT-235
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				xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx																														
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				xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx																														
GP14A F1-V Alhydrogel	CO92	RED	WHITE	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	L	L	I	I	I	I	I	I	I	I		22216B396D/LT-131
				I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	L	L	I	I	I	I	I	I	I	I		221B37023F/LT-132
				I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	L	L	I	I	I	I	I	I	I	I		222258140A/LT-133
				I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	L	L	I	I	I	I	I	I	I	I		22222E6823/LT-134
				I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	L	L	I	I	I	I	I	I	I	I		2221431C17/LT-135
				I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	L	L	I	I	I	I	I	I	I	I		221D757C7E/LT-136
				I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	L	L	I	I	I	I	I	I	I	I		2222562B00/LT-137
			BLACK	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	L	L	I	I	I	I	I	I	I	I		2221754939/LT-138
				I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	L	L	I	I	I	I	I	I	I	I		221D5B0C51/LT-139
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GP14B F1-V Alhydrogel	CO92	BLUE	BLACK	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	L	L	I	I	I	I	I	I	I	I		221B2E7B71/LT-236
				I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	L	L	I	I	I	I	I	I	I	I		221D484645/LT-237
				I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	L	L	I	I	I	I	I	I	I	I		22276D3C14/LT-238
				I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	L	L	I	I	I	I	I	I	I	I		221D4C655D/LT-239
				I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	L	L	I	I	I	I	I	I	I	I		2222516E3F/LT-240
				xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx																														
				xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx																														
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				xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx																														
GP15	CO92	BLUE	WHITE	DIED — DURING CHALLENGE																														221D481476/LT-141
				I	I	I	S	>	I	I	I	I	I	I	I	I	I	I	I	I	I	I	L	L	I	I	I	I	I	I	I	I		221D697718/LT-142
				I	I	I	S	S	S	D																								~~2228163110/LT-143~~
				I	I	I	S	S	I	I	I	I	I	I	I	I	I	I	I	I	I	I	L	L	I	I	I	I	I	I	I	I		2222526107/LT-144
				I	I	I	S	S	I	I	I	I	I	I	I	I	I	I	I	I	I	I	L	L	I	I	I	I	I	I	I	I		22217D4C57/LT-145
		BLACK	BLACK	I	I	I	S	S	I	I	I	I	I	D																				~~22222E5018/LT-146~~
				I	I	I	S	S	D																									~~221B2C4420/LT-147~~
				I	I	I	S	S	I	I	I	I	I	I	I	I	I	I	I	I	I	I	L	L	I	I	I	I	I	I	I	I		222156603B/LT-148
				I	I	I	S	S	S	S	D																							~~2221522454/LT-149~~
				I	I	S	S	D																										~~22218B412F/LT-150~~
Alhydrogel	CO92	GREEN	WHITE	I	I	I	D																											~~2221477A4F/LT-151~~
				I	I	I	D																											~~2222581133/LT-152~~
				I	I	I	S	D																										~~22223B4F59/LT-153~~
				I	I	I	S	D																										~~2221261D5A/LT-154~~
				I	I	I	S	S	I	I	I	I	I	I	I	I	I	I	I	I	I	I	L	L	I	I	I	I	I	I	I	I		2221793120/LT-155
		GREEN	BLACK	I	I	I	S	S	I	I	I	I	I	I	I	I	I	I	I	I	I	I	L	L	I	I	I	I	I	I	I	I		221D725567/LT-156
				I	I	I	S	D																										~~221D5E4642/LT-157~~
				I	I	I	D																											~~22212C640B/LT-158~~
				DIED — DURING CHALLENGE																														22274C6575/LT-159
				I	I	I	D																											~~22252D2519/LT-160~~

Animal Caretakers: Contact LTC Anderson, Office Ext 4933, Fax Ext 2152, Home (301) 473-5059 if you have any questions

- ... dead animals
- ... chip number of dead mice with scanner
- ... number of animals alive in each cage
- ... after Chip # means the chip has fallen out

HEAD COLOR
WHITE RUN 1 AEROSOL
BLACK RUN 2 AEROSOL

Mouse weights for aerosol challenge of 28 Jun 96

34.2, 32.9, 25.7, 35.5, 40.0, 37.0, 37.4, 31.7, 29.2, 41.0 gm = 34.7 avg gm

The counts were as follows:

Prespray -- 4.2 x 10e9 (48/34/43 on 10e7 plate)

AGI's

#1 -- 2.6 x 10e7 (18/36/25 on 10e5 plate)

#2 -- 2.2 x 10e7 (222/196/229 on 10e4 plate)

fuann

AEIR[illegible]L EXPOSURE SHEET

Aerosol exposure #: 96-030 II Location: 41-4 Date: 28 Jun 96

Aerosol Operator: SPC Holmes Agent: Plague - CO92

Pre-operational Check Performed: ✓ Dry T: 75 Wet T: 68 Rel. Hum.: 70% Protocol #:

Exposure System Type/#: Nose-Only P.I.: LTC Anderson

System Flow Rate: 12 LPM Timer Check: ✓ Pre ✓ Post

Collison #: A Start time 0940 1104

Panel #: 3 End time 0946 1109

Electronic Flow Meter #: E0979 OK

Run #	Animal #	Animal Species	Start T Dry	Start T Wet	Start T Rel. Hum.	5 min T Dry	5 min T Wet	5 min T Rel. Hum.	Start Time	AGI #	Comments
1	24	mice	73	68	77%	74	71	85%	0945	366	1 mouse Dead on Arrival
2	25	mice	73	68	77%	74	71	85%	1031	213	1 Dead mouse during Aerosol

Active Immunization

Date: 28-Jun PI: LTC Anderson

Agent: Plague

Strain: CO92

Animal Model: Mouse

Wt: (Ave.) 34.7g CO92 LD50 = 2.1E+04

Run #	AGI cfu / ml	AGI cfu	Aerosol cfu / l	MV l	Inhaled Dose cfu	LD50s	Plague Strain
1	2.60E+07	2.60E+08	4.33E+06	0.03	1.30E+06	61.90	CO92
2	2.20E+07	2.20E+08	3.67E+06	0.03	1.10E+06	52.38	CO92

Protocol: Long Term F1/V — 25/26 V/F1 ELISA Summary

Exhibit GA18

Bleed: 21JUN96 Plate	Serum	Day: Day +204 Group	TREATMENT	26JUN96 F1 TITER	25JUN96 V TITER
1A	8906	GP13A	10F1+20V	10,240	20,480
1B	8907	GP13A	10F1+20V	1,280	40,960
2A	8908	GP13A	10F1+20V	10,240	81,920
2B	8909	GP13A	10F1+20V	10,240	81,920
3A	8910	GP13A	10F1+20V	5,120	20,480
3B	8911	GP13A	10F1+20V	20,480	20,480
4A	8912	GP13A	10F1+20V	10,240	40,960
4B	8913	GP13A	10F1+20V	40,960	10,240
5A	8914	GP13A	10F1+20V	2,560	81,920
5B	8915	GP13A	10F1+20V	10,240	81,920
6A	8916	GP13B	10F1+20V	5,120	20,480
6B	8917	GP13B	10F1+20V	20,480	81,920
7A	8918	GP13B	10F1+20V	2,560	40,960
7B	8919	GP13B	10F1+20V	20,480	163,840
8A	8920	GP13B	10F1+20V	10,240	81,920
8B	8921	GP14A	30ugF1-V	2,560	81,920
9A	8922	GP14A	30ugF1-V	2,560	81,920
9B	8923	GP14A	30ugF1-V	1,280	40,960
10A	8924	GP14A	30ugF1-V	1,280	40,960
10B	8925	GP14A	30ugF1-V	1,280	81,920
11A	8926	GP14A	30ugF1-V	2,560	81,920
11B	8927	GP14A	30ugF1-V	5,120	81,920
12A	8928	GP14A	30ugF1-V	1,280	40,960
12B	8929	GP14A	30ugF1-V	2,560	163,840
13A	8930	GP14A	30ugF1-V	5,120	81,920
14A	8931	GP14B	30ugF1-V	10,240	163,840
14B	8932	GP14B	30ugF1-V	20,480	81,920
15A	8933	GP14B	30ugF1-V	10,240	655,360
15B	8934	GP14B	30ugF1-V	5,120	81,920
16A	8935	GP14B	30ugF1-V	2,560	40,960
16B	8936	GP15	PlagueUSP	2,560	2,560
17A	8937	GP15	PlagueUSP	2,560	2,560
18A	8938	GP15	PlagueUSP	2,560	2,560
18B	8939	GP15	PlagueUSP	5,120	2,560
19A	8940	GP15	PlagueUSP	2,560	2,560
19B	8941	GP15	PlagueUSP	5,120	2,560
20A	8942	GP15	PlagueUSP	5,120	2,560
20B	8943	GP15	PlagueUSP	20,480	5,120
21A	8944	GP15	PlagueUSP	40,960	640
21B	8945	GP15	PlagueUSP	5,120	1,280
22A	8946	GP16	ALH alone	640	2,560
22B	8947	GP16	ALH alone	320	640
23A	8948	GP16	ALH alone	320	640
23B	8949	GP16	ALH alone	320	1,280
24A	8950	GP16	ALH alone	320	640
24B	8951	GP16	ALH alone	640	640
25A	8952	GP16	ALH alone	320	1,280
25B	8953	GP16	ALH alone	1,280	2,560
26A	8954	GP16	ALH alone	640	640
26B	8955	GP16	ALH alone	1,280	5,120
27A	F1/V	POOL		327,680	1,310,720
27B	Norm Mouse	POOL		320	2,560

V-titer too high, no control serum.

GEOMEAN: Group	TREATMENT	F1 TITER	V TITER
GP13A/B	10F1+20V	8,[illegible]12	44,926
GP14A/B	30ugF1-V	3,[illegible]78	85,794
GP15	PlagueUSP	5,[illegible]87	2,229
GP 16	ALH alone	[illegible]20	1,194

For plague challenge of 5 of 96

140 mo. C092

74 mo. C12

data from Ken Welkos

TWC
8 JUL 96

plague-challenge.sc 7/5/96 7/5/96

P.I. = LTC George Anderson
40 mice, C092 - 100 LD50s
30 mice, C12 - 100 LD50s

Parenteral challenge of mice with C092/M.S. and C12/M.S.

1. Streak 1 slant each with the Master Seed of C092 and C12.
   Incubate 2 days at room temperature.
2. Harvest by suspending in 4-5 mls of HIB.
3. Read OD620 of a 1/10 dilution.
4. Adjust to OD 1.0

7/5/96:
Adjusted ODs and read final ODs on 1/2 dilutions:
Final OD = **1.064**, for C092
" " - **1.10**, for C12

C092/M.S.:

1. Prepare dose
5.0 -7.5x10e2/ml:
   (1) Add 0.2 ml OD 1.0 to 1.8 mls HIB.
   (2) Add 0.2 ml (1) to 1.8 mls HIB.
   (3) Add 0.5 ml of (2) to 4.5 mls HIB.
   (4) Add 0.5 ml of (3) to 4.5 mls HIB.
   (5) Add 0.5 ml of (4) to 4.5 mls HIB.
   (6) Add 4.0 ml of (5) to 36 mls HIB - - Pipet 10 mls into each of 3 tubes:
   mice INOCULUM: 1 x 10e3/ml: ~200 cfu/dose

---

2. Plating: The sample will be diluted in HIB and plated on SBAP:

suspension	Conc./ID	dilution	Total No. no. plates	plates
mice Inoculum	5x10e2/ml	undil, 10-1	5 each	10

RESULTS:
7/5/96 doses: 1.4 x 10e3/ml, 280 **cfu/dose** (**140 LD50s**)

7/12/96 doses: 6.5 x 10e2/ml, 130 **cfu/dose** (**72 LD50s**)
7/18/96 doses: ____ x 10e2/ml, ____ **cfu/dose** (____ **LD50s**) - canceled

C12/M.S.:

1. Adjust slant suspension to OD620 = 1.0.
Prepare dose
2.3 x10e3/ml:

(1) Add 0.2 ml OD 1.0 to 1.8 mls HIB.
(2) Add 0.2 ml (1) to 1.8 mls HIB.
(3) Add 0.5 ml of (2) to 4.5 mls HIB.
(4) Add 0.5 ml of (3) to 4.5 mls HIB.
(5) Add 1.0 ml of (4) to 9.0 mls HIB.
(6) Add 6.0 ml of (5) to 18 mls HIB (1/4) - -

INOCULUM, C12-100 sc LD_{50}s (910 cfu). Pipet 10 mls into each of **2 tubes:**
1 x 10e3/ml: ~200 cfu/dose

---

2. Plating: The Inoculum will be diluted in HIB and plated on SBAP:

suspension	Conc./ID	dilution	no. plates	Total No. plates
C12 **Inoculum**	2.3x10e3/ml	undil.	5	
		10-1	5	
		10-2	5	TOTAL-15

RESULTS:

7/5/96 doses: 3.36 x 10e3/ml, **6.7 x 10e2 cfu/dose** (**73.6 LD50s**)
7/12/96 doses: 3.0 x 10e3/ml, **5.8 x 10e2 cfu/dose** (**63.7 LD50s**)
7/18/96 doses: ____ x 10e3/ml, **____ x 10e2 cfu/dose** (**____ LD50s**) – canceled

Exhibit GA19

AIBS PEER REVIEW TO USAMRMC MEDICAL BIOLOGICAL DEFENSE RESEARCH PROGRAM ON PLAGUE

REVIEW PANEL

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APPROVED: _____ DATE: March 12, 1996
Tom Schwan, PhD
KATHLEEN McDONOUGH, P.D.

INTRODUCTION

AIBS was requested by US Army Medical Research and Development Command (USAMRDC) to convene a review Panel to provide an assessment of the scientific merit of the Medical Biological Defense Research Program (MBDRP) on Plague. It was requested that the three scientific reviewers have a collective knowledge of the following subject areas: *Yersinia pestis*, Vaccine Production, Molecular Genetics and FDA requirements for a vaccine. Such a panel was convened and provided with documentation by USAMRDC to read prior to the conference. This consisted of abstracts prepared by the individual investigators who form the MBDRP on Plague (see Appendix 1.)

CHARGE TO PANEL

Three scientific reviewers were asked to evaluate the MBDRP on Plague. They independently reviewed material provided by USAMRDC and attended a conference on the subject matter. They were asked to judge the scientific merits of the Program.

The reviewers, individually, provided comments to AIBS, who in turn compiled this written report summarizing these comments and the discussions at the conference. The Chairman of the Review Panel read and approved the report prior to its submission to USAMRDC.

PRESENTATION SUMMARIES

The conference comprised presentations by each of the following investigators. Abstracts were provided for by each and are attached as Appendix 1.

COL ARTHUR FRIEDLANDER
Overview of plague program

COL RUSSELL BYRNE
Antibiotic treatment of experimental pneumonic plague

DR. PATRICIA WORSHAM, DR. M. LOUISE PITT, LTC KELLY DAVIS
F1 is not a required virulence factor for the mouse or non-human primate

MAJ GERALD P. ANDREWS, LTC GEORGE J. ANDERSON, JR.
Protective efficacy of active immunization with purified F1 from *Yersinia pestis* and an *Escherichia coli* recombinant strain against lethal parenteral and respiratory plague challenge

DR. PATRICIA WORSHAM
Studies on the role of the pigmentation locus in the pathogenesis of *Y. pestis*

DR. SUSAN L. WELKOS, LTC KELLY J. DAVIS
Analysis of the role of pPst encoded genes in pathogenesis of infection by *Y. pestis*

DR. ALAN SAMPLE
Plasminogen activator protease degrades proinflammatory cytokines

MAJ GERALD P. ANDREWS, DR. SUSAN STRALEY, DR. ALAN SAMPLE, MAJ GERALD P. ANDREWS
Cloning, Expression, Purification, and Protective Efficacy of Yops and pH 6 antigen

LTC GEORGE J. ANDERSON, JR., DR. DAVID HEATH
Cloning, expression, and protective efficacy of V antigen

LTC GEORGE J. ANDERSON, JR.
Cloning, expression, and protective efficacy of F1-V fusion protein

DR. JEFFREY PULLEN
Determination of important B and T-lymphocyte epitopes in the F1 and V antigen proteins of *Yersinia pestis*

COL ARTHUR FRIEDLANDER
Overview of future plans

SUMMARY EVALUATIONS OF THE RESEARCH AREAS

The review panel read the abstracts provided by the investigators prior to the meeting on February 15, 1996, and listened to presentations by each of the investigators at the meeting. The following comments include recommendations to individual investigators, and are intended to be constructive. Certain points apply to more than one project, or even to the program as a whole, and hence may appear repetitive. Also, the reviewers recognize that some of their recommendations may be affected by programmatic decisions that are beyond the control of the immediate Program staff and thus may not prove to be possible.

COL ARTHUR FRIEDLANDER
Overview of plague program

The USAMRMC Plague Research Program's primary objective is to develop a vaccine that will protect military personnel if exposed to an aerosol attack of *Yersinia pestis*, the causative agent of plague. Given that the currently available vaccine (USP) protects primarily through anti-F1 antibody, that this vaccine offers very poor protection from primary pneumonic plague, and that $F1^-$ strains are highly virulent, there clearly is a need for a new, more protective vaccine. Once developed, the general population living in areas endemic for plague would also benefit from such a vaccine.

Most of the projects presented as separate studies and presentations clearly meet the program's primary objective. Part of the rationale for the approach taken is that an aerosol attack of *Yersinia pestis* might include strains that do not produce the F1 capsular antigen. Given that the current vaccine (USP) stimulates primarily antibodies

AIBS PEER REVIEW TO USAMRDC
MEDICAL BIOLOGICAL DEFENSE RESEARCH PROGRAM
ON PLAGUE

TIME: February 15, 1996, 8.00am to 5.00 pm

LOCATION: US Army Medical Research Institute of Infectious Diseases, Conference Room, Fort Detrick, Frederick, MD

EXECUTIVE SUMMARY

Overall, the program has made very significant and impressive advances in only a few years towards the development of a new vaccine, and Dr. Friedlander and his entire team of investigators can be proud of their accomplishments to date. They clearly have a very viable, sound program with a good team of investigators that is focused with high potential to succeed. It is hoped that the administration will continue to support this effort and provide the group with the resources and time necessary to complete their task. The investigators clearly considered the recommendations of the previous reviewers and incorporated several of the suggestions into their program.

The team has invested significant effort in examining numerous virulence determinants of *Yersinia pestis* for their ability to stimulate protection through immunization. The F1 capsular antigen and the V antigen have been shown by investigators in other laboratories to be good candidates for inclusion in a new multivalent subunit vaccine. The team at USAMRMC has confirmed the protective value of these two antigens. However, realizing that F1 and V antigens might not be sufficient for full protection against all virulent strains of *Y. pestis*, the group has worked through an impressive list of additional candidates. The only other antigen that offered significant protection was YopD, although protection was only observed when mice were challenged with the $F1^-$ strain. Passive immunizations with anti-F1 and anti-YopM antisera deserve further attention. Combined antibiotic treatment and immunization might increase the survival of animals challenged by aerosol.

The team appears to make use of mice and nonhuman primates as excellent animal models for both their parenteral and aerosol challenge experiments. The current vaccine study protocols for test challenges are very good.

The development of *in vitro* correlates of immunity should be a high priority of the program. It is currently the weakest portion of the future plans. As discussed with the investigators, the assumption that protection is solely antibody-mediated has potential difficulties. Before continuing studies to map active B cell epitopes, the investigators need to determine the role of T cells in immunity to plague.

examined for *Y. pestis* in the LD50 studies and survivors were examined for clearance of the organisms to determine the full level of protection provided by vaccination.

In the first study, the V antigen was examined for its ability to generate a protective immune response in mice challenged by parenteral subcutaneous or aerosol challenge with either the $F1^+$ or $F1^-$ isogenic strains of *Y. pestis*. Recombinant V antigen was cloned and expressed in two fusion/expression systems and used with an adjuvant approved for human use (Alhydrogel). Both preparations of rV antigen were administered twice and provided very good protection in mice challenged by both routes and both strains. This is an excellent study and identifies (as another laboratory has demonstrated independently) the V antigen as an excellent candidate immunogen to include in a vaccine to protect from aerosol infections with either $F1^+$ or $F1^-$ strains. These studies are critical to the program's objective and provides some quite exciting results.

The second study extends the work on the V antigen of *Y. pestis* by examining protection following a single dose of 10 μg (the previous study used two immunizations prior to challenge). Mice were subsequently challenged by aerosol exposure to either low and high doses of the $F1^+$ or $F1^-$ strain. Protection ranged from 70% to 78% survival in these mice, demonstrating that a single immunization could afford significant protection from an aerosol route of infection. However, the schedule including a primary immunization followed by a single boost afforded 20% to 30% greater protection (previous report). While it is of interest what level of protection results from a single dose, future work with nonhuman primates will likely confirm what we know about many other bacterial vaccines, i.e., better protection results with boosts following the primary immunization.

Two areas need to be addressed in future work on the V antigen. The studies presented used the V antigen tagged with histidine from the pET vector. If this antigen is to be used in humans, a method for the efficient removal of the his-tag is needed. Identifying the active sites on the V antigen responsible for protective immunity as well as potential negative biological activities, such as immune suppression, may be required for this antigen to be safe. The group might also consider examining how long protective immunity lasts following vaccination with the V antigen. Some of these issues were addressed by Dr. Friedlander in his closing remarks.

LTC GEORGE J. ANDERSON, JR.
Cloning, expression, and protective efficacy of F1-V fusion protein (abstract 17)

Prior studies have confirmed the potential for both F1 and V antigen to protect mice from *Y. pestis* by both parenteral and aerosol routes. In this study a construct was made containing the F1 and V antigen genes for expression of a fusion protein. When the F1-V fusion protein was used for immunization, mice were protected when challenged by needle or aerosol with either the F1 positive or F1 negative strain of *Y. pestis*. Poorer protection resulted when only a portion of the V antigen was expressed as a fusion protein with F1. This work is quite clever and interesting, and advances the program's effort towards the development of a multivalent vaccine. The attempt to make fusions of these two antigens also demonstrates an advance towards reducing

the steps required for making and purifying antigens for the vaccine. The investigators are also testing longer term antibody responses and how long protection lasts (a concern raised from the previous studies with the V antigen alone). Antibody responses to the F1 and V antigen components of the fusion protein were also examined. Both F1 and the V antigen have been shown by other workers to be protective and now the group at USAMRMC has shown that rF1 and rV are the best candidates identified to date for a new plague vaccine.

Again, this fusion protein has a histidine tag, which will need to be removed prior to its use in humans.

DR. JEFFREY PULLEN
Determination of important B and T-lymphocyte epitopes in the F1 and V antigen proteins of *Yersinia pestis* (abstract 18)

This study attempts to identify important B and T cell epitopes within both the F1 and V antigens, however only B cells were addressed in the presentation. Identifying the functional epitopes in these proteins is important both to an understanding of the protective mechanisms stimulated by these two immunogens, and for assessing the potential of using synthetic peptides rather than entire recombinant proteins in a vaccine. This study is an important part of fulfilling the long-term objective of developing a useful vaccine. However, the usefulness of the current appproach should be carefully reconsidered.

The use of short peptides to generate antibodies without conjugation to carrier molecules has, in general, not been very successful. Although it is sometimes possible to generate antibodies against short peptides, it is unlikely that the response will be protective without some T cell involvement. The investigators' initial experiments showed that peptides generated from the region of the protein known to be antigenic failed to generate a protective response despite generating significant antibody production. These results should have alerted them to the problems inherent in this approach. Instead, the investigators expanded their studies in response to these findings by making and testing additional peptides covering the whole of V antigen and F1 protein. This was a lot of work, using a lot of mice, that generated very little useful information. A simpler and more direct approach to begin mapping the reactive epitopes in these immunogens is to screen the overlapping peptides *in vitro* using antisera from animals or humans that have either had infections with *Y. pestis* or been immunized with native F1 and/or V antigen. Another concern is that in the future goals, it was stated that the response to the peptides, rather than to the native antigen will be tested to better determine the response. However, since the goal is to get protective antibodies, it seems that the reponse to native antigen, which is what the animals will see in an actual infection, is what should be measured.

It is also important for the investigators to determine the nature of a protective immune response to *Y. pestis* infection before restricting their focus and undertaking such labor-intensive studies to define only B cell epitopes. Antibody reactivity does not assure protection, and with some pathogens high antibody titers have even been correlated with disease progression. In addition, non-F1 antigens may evoke a

COMBINED RECOMMENDATIONS AND CONCLUSIONS

The USAMRMC's program to develop a new subunit vaccine for pneumonic plague has been very productive and has made significant advances towards this objective. The leader and research team are highly skilled, competent investigators and, with continued support, it is anticipated that a new vaccine for human trials is only a few years away. The investigators have used very effective immunization and challenge protocols to test immunogens in both mice and nonhuman primates for protection against plague following either parenteral or aerosol exposures to *Yersinia pestis.* Having the facilities to safely execute aerosol transmission studies is a critical component of this program. The team has confirmed and extended the data supporting the potential for both recombinant F1 and V antigens to afford significant protection. The work using the F1-V antigen fusion protein is exciting and represents a significant advance made by this team.

The team has examined numerous other antigens for identifying additional protective immunogens, especially for challenge with strains of *Y. pestis* lacking the F1 antigen. For such isolates, the V antigen and possibly YopD are the only useful candidates identified to date. The addition of one more antigen would likely solve the problem of non-responders, as well as strengthen the response in all individuals. The choice of antigens being tested for potential vaccine components appears somewhat random. These studies could be focused better by determining what proteins induce an immune response, thereby demonstrating which determinants are most likely being seen by the immune system. Although it is not possible to predict in advance which antigens are protective, the search could have been directed more towards antigens known to induce an antibody response in infected human patients and laboratory infected animals. Additional focus on the basis of immunity to plague challenge is also recommended. The investigators are also aware of the immunosuppressive effects of V antigen, and plan to examine the mechanisms involved. These types of studies should allow the team to "fine tune" the V antigen to increase its efficacy and safety as a vaccine component.

The development of *in vitro* correlates of immunity should be a high priority of the program and is currently the weakest area of the future plans. As discussed with the investigators, the assumption that protection is solely mediated by antibody has potential difficulties. Before continuing studies to determine important B cell epitopes, the role of T cells needs to be addressed in collaboration with immunologists. There are standard methods, such as adoptive transfer, to determine if T cells protect against challenge with *Y. pestis.* There are also *in vitro* techniques to determine if T cells taken from an immunized animal proliferate in response to specific antigens. The studies using synthetic peptides have potential, but this work needs to be done with conjugated peptides. Alternatively, peptides could be attached to larger inert particles that could be taken up by B cells or macrophages that then present the antigen on class II MHC molecules on their surface. Epitope mapping of the F1 and V antigen peptides using immune sera from natural infections would have been an appropriate first step.

APPENDICES

APPENDIX 1: **AGENDA**

APPENDIX 2: **ABSTRACTS**

REVIEW OF PLAGUE RESEARCH PROGRAM

USAMRIID

15 FEBRUARY 1996

0815-0830 Welcome and introduction
COL David Franz, DVM, Ph.D.

0830-0900 Overview of Plague Program
COL Arthur M. Friedlander, M.D.

Treatment

0900-0930 Antibiotic treatment of experimental pneumonic plague
COL Russell Byrne, M.D.

Role of F1 Capsule in Pathogenesis and Immunity

0930-1000 Protective efficacy of active immunization with purified F1 from *Yersinia pestis* and an *Escherichia coli* recombinant strain against lethal parenteral and respiratory plague challenge
MAJ Gerard P. Andrews, Ph.D.

1000-1015 Coffee Break

1015-1100 F1 capsule is not a required virulence factor for the mouse or non-human primate
Patricia L. Worsham, Ph.D.
M. Louise Pitt, Ph.D.
LTC Kelly J. Davis, DVM

Role of Non-F1 Proteins in Pathogenesis and Immunity

1100-1130 Studies on the role of the pigmentation locus in the pathogenesis of *Y. pestis*
Patricia L. Worsham, Ph.D.

1130-1300 Lunch

1300-1320 Analysis of the role of pPst encoded genes in pathogenesis of infection by *Y. pestis*
Susan L. Welkos, Ph.D.

1320-1335 Plasminogen activator protease degrades proinflammatory cytokines
Allen Sample, Ph.D.

1335-1405 Cloning, expression, and protective efficacy of Yops and pH 6 antigen
MAJ Gerard Andrews, Ph.D.

1405-1420 Cloning, expression, and protective efficacy of V antigen
LTC George J. Anderson, Jr., Ph.D.

1420-1435 Cloning, expression, and protective efficacy of F1-V fusion protein
LTC George J. Anderson, Jr., Ph.D.

1435-1450 Determination of important B and T-lymphocyte epitopes in the F1 and V antigen proteins of *Y. pestis*
Jeffrey Pullen, Ph.D.

1450-1515 Overview of future plans
COL Arthur M. Friedlander, M.D.

#17

Recombinant F1-V (rF1-V) Fusion Protein Protects against Lethal Wildtype Yersinia pestis in a Mouse Model

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Bacteriology Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD.

The virulence of F1- strains and their occurence in nature imply that F1 immunogen will not be sufficient for an optimal new plague vaccine. A fusion protein has the theoretical possibility of simplifying and reducing the cost of production of multiple antigens in addition to stabilizing the protein. For these reasons, we developed a fusion protein consisting of both the F1 and V antigens (1). The first fusion protein made consisted of F1 fused with residues 168-175 of the V antigen, a segment which previous studies suggested to contain a protective epitope. This fusion protein was used with the adjuvant alhydrogel (aluminum hydroxide) to immunize female Swiss Webster (Hsd:ND4) mice subcutaneously (s.c.) on days 0 and 30 followed by a s.c. or aerosol challenge with either the F1- C12 strain (LD50 = 9.1 CFU, s.c.; LD50 = 1.1 x 105 CFU, aerosol route) or the wild-type F1+ CO92 (LD50 = 1.9 CFU, s.c. route; 2.1 x 104 CFU, aerosol route) strain of Y. pestis. Endotoxin had been removed from the preparations prior to immunization, so that this would not be a confounding factor.

When 18.5 µg of the F1-V168-275 fusion protein was used to immunize mice, there was 90% survival (9/10) when challenged s.c. with 63 LD50 of the F1+ CO92 strain. The positive control was a group of mice immunized with 10 µg of rF1 which is equivalent to the F1 content of the F1-V168-275 protein. The rF1 control resulted in 100% (10/10) protection. The F1-ELISA IgG titers were the same (1:81920). All mice in alhydrogel control group died (0/9; MTD ± SD, 5.2 ± 1.0). When the F1-V168-275 immunized mice were challenged with 104 LD50 by the aerosol route, protection was 80% (8/10; MTD ± SD, 20.3 ± 7.1) compared to 0% for the control group (0/10; MTD ± SD, 3.1 ± 0.3; 80-104 LD50). The group immunized with rF1 resulted in 70% protection (7/10; MTD ± SD, 9.0 ± 1.0) when challenged with 80 LD50. The addition of part of the V protein onto the F1 protein did not appear to effect its antigenicity.

The F1- strain, C12, was used to test the ability of the partial V portion of the F1-V168-275 protein to protect mice against a lethal challenge. Here 27 µg of the F1-V168-275 fusion protein was used, which is equivalent to 10 µg of the V protein

known to be protective. A s.c. challenge dose of 55 LD50 resulted in 30% survival (3/10, MTD ± SD, 9.4 ± 7.0). All of the controls died (0/10, MTD ± SD, 10.8 ± 4.8). While there was some protection, there was no increase in the MTD. There was a good V-ELISA antibody response to the F1-V168-275 (1:163840). In case this response was not sufficient, another group was immunized with 27 µg, but with complete Freund's adjuvant (CFA). In this case, protection was only 20% (2/10, MTD ± SD, 9.1 ± 3.2), while 10 µg of rV in CFA resulted in 100% protection. The V-ELISA titer when CFA was used was 1:1310720 for F1-V168-275 and rV. A 10-fold increase in the V-antibody titer did not have any effect on protection and the V-ELISA titer was not indicative of protection. When a group of F1-V168-275 mice were challenged with 95 LD50, C12, by the aerosol route, no mice survived (0/10, MTD ± SD, 3.5 ± 0.5) All of the alhydrogel control group died (0/10, MTD ± SD, 3.4 ± 0.5). In other experiments, rV itself gave 80-90% protection against an aerosol challenge.

These results demonstrated the feasibility of making a F1-V fusion protein. The efficacy of F1 was not altered by making a fusion protein. However, while the V168-275 protein portion of the fusion protein was antigenic, it was not immunogenic. This caused us to address the question as to whether the entire V protein could be fused to F1 and whether it would be immunogenic.

Using a fusion protein which combines the whole F1 and the whole V protein (rF1-V) to immunize mice on days 0 and 30 increased the protection afforded by the V portion of the fusion protein. When 13.6 ug of rF1-V was used to immunize mice, there was 100% (10/10) protection against a s.c. challenge of 57 LD50 and 90% (9/10) protection against 1.1 x 106 LD50 C12 strain. Ten micrograms (10 µg) of rV also gave 90% (9/10) protection against 1.1 x 106 LD50, C12 strain. All of the alhydrogel control group died (0/10, MTD ± SD, 6.0 ± 0.0). The rF1-V protein also offered protection against an aerosol challenge. The same immunization schedule resulted in 100% (10/10) when mice were challenge with 546-636 LD50, C12 strain on day 73 postimmunization. When mice immunized with the rF1-V fusion protein were challenged with 762 LD50 of the F1+, CO92 strain by the aerosol route, 100% (10/10) of the mice survived. The F1-V fusion protein was able to protect mice from a significant aerosol challenge from either a F1+ or F1- lethal strain of Y. pestis. This protection is better than the protection afforded by the current Plague Vaccine USP. When mice which were immunized on day 0 and 30 with 0.2 ml of the current vaccine and challenge by the aerosol route on day 73 postimmunization with 546-636 LD50, C12 strain, all of the mice died (0/8, MTD ± SD, 3.3 ± 0.5). The V-ELISA titer to the Plague

Vaccine USP was <1:640.

The recombinant rF1-V fusion protein was produced in E. coli and contained a polyhistidine tag which aids in the purification of the fusion protein. While this protein has been shown to be highly efficacious in the mouse model, it remains to be seen whether this level of protection will be seen the in the non-human primate model. Further, the regulatory issue of whether a histidine tagged protein will be acceptable to the Food and Drug Administration needs to be resolved.

1. Heath, D.G., G.W. Anderson, Jr., J. M. Mauro, S.L. Welkos, and A.M. Friedlander. Protection against experimental bubonic and pneumonic plague by a recombinant capsular F1-V antigen fusion protein vaccine. manuscript submitted.

2. Brubaker, R.R., A.K. Sample, D.Z. Yu, R.J. Zahorchak, P. C. Hu, and J.M. Fowler. 1987. Proteolysis of V antigen from Yersinia pestis. Microbial. Pathogenesis. 2:49-62.